

Monograph on Gum Arabic

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MONOGRAPH
ON
GUM ARABIC

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GUM ARABIC

Table of Contents

Summary	1
Chemical Information	3
Biological Data	8
Biochemical Aspects	11
Master Bibliography	19
Bibliography of Documents Not Included in Monograph	21
Documents	49

GUM ARABIC

Summary

According to Monke (16), gum arabic administered orally to rats is excreted unchanged in the feces. However, Shue (19, 20) found the digestability of gum arabic in rats to be as high as 80%, while a figure of 71% was determined by Booth (5). In a study by O'Dell (17), guinea pigs were observed to digest 90% of the administered dose.

It has been demonstrated that gum arabic has the ability to inhibit the action of certain esterases (9).

Although there are no standard long-term feeding studies available on the toxicological effects of gum arabic, several nutritional studies with rats (5, 7), guinea pigs (4), and rabbits (14) reveal no toxic effects attributable to gum arabic at levels of 15-20% of the diet.

Booth, et. al. (5), administered gum arabic at a 15% dietary level to test rats for 62 days and found that growth was enhanced. No toxic effects were reported. Drinker, et. al. (7), provided a 3.5% solution of gum arabic to rats as a sole liquid source for 34 weeks. Again, no demonstrable toxic effects were reported.

Booth, et. al. (4), fed a variety of gum arabic diets comprised of 15% gum arabic to guinea pigs over a period of 6-9 weeks. No toxic effects were shown.

Hove and Herndon (14) fed a 20% gum arabic diet to New Zealand rabbits for 40 days. Animals fed a gum arabic diet showed an elevated average weight in comparison to controls. No toxic effects were evident.

A number of workers have investigated and compiled reports on allergic reactions in humans to gum arabic (3, 11, 12). A majority of the reported cases have involved occupational exposure (printers, hair-dressers, and confectioners). Gelfand (12) has shown in sensitized individuals that oral administration of gum arabic results in clinical manifestations.

GUM ARABIC

Chemical Information

I. Nomenclature

- A. Common Names
 - 1. Gum Arabic
 - 2. Acacia
- B. Chemical Names
 - A complex polysaccharide
- C. No Trade Names
- D. CAS Registry Number -- PM 9000015

II. Empirical Formula

Gum arabic is a gum exudate which is a metal (Ca, Mg, K) salt of a polysaccharide acid, arabic acid. Analysis shows it to be composed of 30.3% L-arabinose, 36.8% D-galactose, 11.4% L-rhamnose and 13.8% glucuronic acid (1).

III. Structural Formula

The structures of gum arabic and other acacia gums (1) may be considered in terms of: the galactan core, the nature of the hexuronic acid, residues and their modes of linkage to those of D-galactose, the nature of the peripheral chains of L-Arabinofuranosyl and associated sugar residues, and the location of L-rhamnopyranosyl residues (when present).

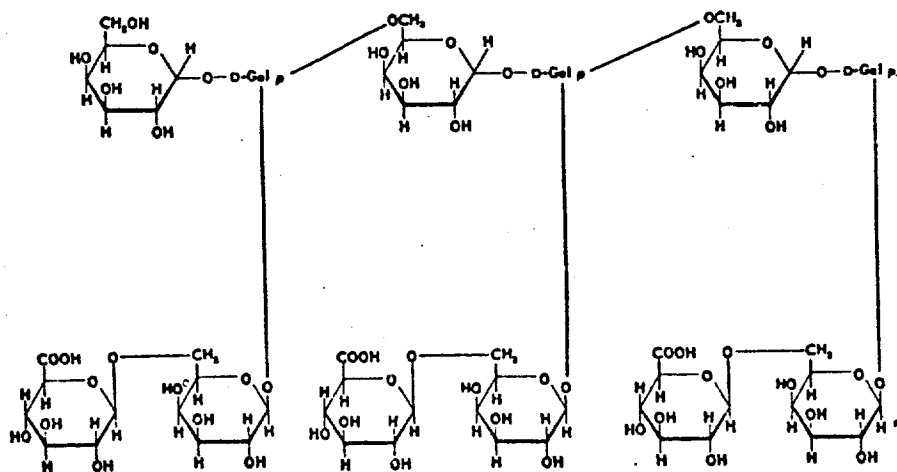
Table I shows the oligosaccharides that have been adequately characterized as partial fragmentation products (1).

TABLE I
Oligosaccharides from Partial Hydrolysis of Acacia Gums

Oligosaccharides	Acacia gums						
	<i>senegal</i>	<i>pycnantha</i>	<i>meurnsii</i>	<i>arabica</i>	<i>nubica</i>	<i>nilotica</i>	<i>drepanolobium</i>
α -D-Galp-(1 \rightarrow 3)-L-Ara (1)	+						
β -L-Arap-(1 \rightarrow 3)-L-Ara (2)	+		+	+	+	+	+
β -L-Araf-(1 \rightarrow 3)-L-Ara (3)		+		+	+		+
β -L-Araf-(1 \rightarrow 2)-L-Ara (4)						+	
β -D-Galp-(1 \rightarrow 3)-D-Gal (5)	+	+	+	+	+	<i>a</i>	+
β -D-Galp-(1 \rightarrow 6)-D-Gal (6)	+	<i>a</i>	+	+	+	+	+
β -D-GpA-(1 \rightarrow 6)-D-Gal (7)	+	+	+	<i>a</i>	+	+	+
4-Me- β -D-GpA-(1 \rightarrow 6)-D-Gal (8)	<i>a</i>		+	<i>a</i>		+	+
α -D-GpA-(1 \rightarrow 4)-D-Galp (9)				<i>a</i>	+	+	+
4-Me- α -D-GpA-(1 \rightarrow 4)-D-Galp (10)				<i>d</i>		+	+

^aOligosaccharides characterized by paper chromatography only, or presence of linkage inferred from other data (for example, cleavage products from methylated gum or derivative), or both.

Methylation and degradation studies of gum arabic (*Acacia senegal*) along with periodate oxidation and other confirmatory reactions have lead to the following proposed structure (20):



IV. Molecular Weight

In 1970, Churms and Stephen (6) obtained a value of 310,000 for the molecular weight of gum arabic. Other sources (1, 13, 22) report values of 220,000, 240,000 and from 240,000 to 300,000.

V. Specifications

A. Chemical -- USP Grade

Loss on drying	10.0% max.
Total ash	4.0% max.
Arsenic	3 ppm max.
Heavy metals	40 ppm max.

B. Food Grade

see A. above

C. Food Chemicals Codex

Limits of Impurities

Arsenic	Not more than 3 ppm
Ash (total)	Not more than 4%
Ash (acid insol.)	Not more than 0.5%
Heavy metals	Not more than 10 ppm (0.004%)
Insoluble matter	Not more than 1%
Lead	Not more than 10 ppm (0.001%)
Loss on drying	Not more than 15%

VI. Description

A. General Characteristics

1. Natural - light yellow gum exuded from wounds in bark of various species of the genus Acacia as spheroidal tears up to 32 mm in diameter
2. Commercial - white powder or flakes, odorless and tasteless

B. Physical Properties

1. Specific gravity 1.35 to 1.49
2. Solubility
 - a. insoluble in alcohol and most organic solvents
 - b. soluble in water (37 g/100 g solution at 25 deg.), glycerol and propylene glycol
 - c. aqueous solution acid to litmus (pH 4.5-5.5)
 - d. viscosity of solutions relatively low (200 cps for 30% solution)
3. Stability

Stable in sealed containers

VII. Analytical Methods

Gum arabic occurs in many commercial products. In order to identify it and determine the amount present, it must first be isolated. In simple cases, the mixture is heated with dilute acetic acid, filtered, cooled and treated with ethanol or acetone to precipitate the gum (1).

When the gum is isolated, it can be determined qualitatively by a systematic analytical scheme such as one devised by Ewart and Chapman (8). This method is based on precipitation reactions with calcium chloride, sodium hydroxide, barium hydroxide and lead acetate. This scheme distinguishes between pectin, alginate, gelatin, starch, carboxymethylcellulose, methylcellulose, and several other gums, including carrageenan, tragacanth, agar, locust, karaya, ghatti.

Another procedure for the isolation and detection of gum arabic is to reflux the sample in 50 ml water and 50 ml of 10% by volume sulfuric acid for 3 hours. Then 3 g of barium hydroxide in 100 ml of water is added, the pH adjusted to 7, evaporated to a small volume (2). The solution is then paper chromatographed with butanol: pyridine: water (3:2:1.5) as the mobile solvent and phthalic acid-aniline and naphthoresorcinol-trichloroacetic acid as developing colors. In addition to gum arabic, this method can be used to separate and identify other gums including carob bean, tragacanth, several sugars, and pectin.

Padmoyo and Miserez (19) have used microelectrophoresis to separate and identify the gums: arabic, tragacanth, carrageenan, carob and guar, as well as, gelatin, pectin starch, dextrin, agar, sodium alginate, methylcellulose and carboxymethylcellulose. The electrophoresis is carried out on cellulose acetate strips. Substances with similar motility are differentiated by staining.

VIII. Occurrence

Gum arabic is obtained from trees belonging to the various species of the genus Acacia. There are about 500 species located in tropical and subtropical areas. Almost all of the gum used in the United States is imported from the Sudan Republic and is from the species Acacia senegal.

GUM ARABIC

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GUM ARABIC

Biological Data

I. Acute Toxicity

None available

II. Short-term Studies

Rats

A control group of weanling rats was fed a basal ration. Five test groups were fed basal rations in which a supplement of gum arabic of 0.25, 0.5, 1, 2, and 4 g/5 g of ration was added. Animals were weighed, and feces were collected, weighed, and determined for nitrogen (20).

At a dietary level of 16% gum arabic, rats showed a weight gain of 75% of control animals. Digestibility data indicated that approximately 80% of gum arabic was absorbed (4). No toxic effects were observed.

Two groups of 6 weanling male rats were fed: one group a basal diet for 62 days, the other a diet containing 15% gum arabic for 62 days. The group fed gum arabic showed a mean weight gain of 224 g/rat; the control group showed a mean weight gain of 199 g/rat. There were no toxic effects shown (5).

In another experiment, two groups of 5 rats were both fed 5 g/day of a basal diet; one group had a supplement of 0.75 g/day of gum arabic added to the diet for 7 days. On day 8 and 9, this supplement was omitted. Animals fed gum arabic showed an increase of 18 g/animal over the control group. Digestibility (Intake - Increase in fecal weight/Intake) was calculated to be 71% of the control (sucrose) (5).

Six male Wistar rats were given a 3.5% solution of gum arabic as a sole liquid source for a period of 34 weeks. These animals were fed a stock diet and were provided the gum arabic solution ad lib. One of these rats began the regimen at 12 weeks of age; the other 5 began at 22 weeks of age (7).

During the course of the experiment, each rat was weighed weekly. Shortly before termination of the experiment, hematologic (RBC, WBC, and hemoglobin) determinations were made on the test animals (7).

Weight gain was normal as were the hematologic indices. No toxic effects of any kind were evident (7).

Unfortunately, insufficient data were reported to determine a mg/kg/day dosage level of gum arabic.

Guinea Pigs

Two groups of guinea pigs used as controls were fed a Rockland stock diet. The group of 30 animals fed 6 weeks gained an average of 6.9 g/day; the other group of 4 animals fed 9 weeks gained an average of 6.6 g/day. In order to test the effects of various synthetic diets, a basal diet was formulated and various supplements added. Twenty-two of these diets contained 15% gum arabic and 1 contained 20% gum arabic. These diets were fed to a total of 133 animals for periods of 3 to 9 weeks. Average weight gain/day varied from 2.8 to 7.0 g depending on constituents of the diet. These gum arabic diets enhanced growth to a greater extent than diets using bulking agents other than gum arabic. In no case were toxic effects attributable to gum arabic observed (4).

Rabbits

Two groups of 4 New Zealand-white rabbits were fed ad lib for 40 days. One group was fed a diet consisting of 20% casein, 51% sucrose, and 10% cellulose; a second group was fed a diet consisting of 20% casein, 31% sucrose, 10% cellulose, and 20% gum arabic. Rabbits fed the gum arabic diet had a mean daily weight gain of 16.7 g; control animals had an 11.5 g gain (14).

III. Long-term Studies

None

IV. Special Studies

Sensitization

Gelfand examined 10 subjects suffering from allergic disorders and, by a combination of skin and serologic tests as well as clinical trial and elimination procedures, confirmed gums (gum arabic, gum tragacanth, and gum karaya) as the source of allergic disorders. Clinical trials in which these gums were given orally produced the same symptomology. In these trials, gum arabic was definitely implicated as a cause of clinical manifestations in man (11).

Fetotoxic

A 1% aqueous suspension of gum arabic when injected intraperitoneally daily between the 11th and 15th day of gestation in NMRI mice produced no fetotoxic effects (10).

GUM ARABIC

Bibliography - Biological Data

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GUM ARABIC

Biochemical Aspects

I. Breakdown

Gum arabic contains an oxidase-type enzyme which renders it unsuitable for use in pharmaceutical preparations containing easily oxidizable active constituents (15).

II. Absorption - Distribution

Monke (16) fasted 20 young male rats for 48 hours, then gave them 10 g of a mixture containing 34% of white powdered gum arabic and 66% of cacao butter. After 72 hours, the rats were anesthetized and their livers were removed and tested for glycogen level. The difference in liver glycogen between the control and the gum arabic fed rats was found to be insignificant. Monke therefore concluded that no part of the gum arabic molecule is subject to disintegration by the enzymes of the digestive tract of the rat.

Later studies indicate that gum arabic is partially digested by the rat. Weight gains and food efficiencies were determined with groups of 6 rats fed 15% gum arabic for 62 days. The food efficiency was identical for the experimental and control groups. However, the animals on gum arabic had a mean weight gain of 224 g vs 199 g for the controls. In another study with groups of 5 rats pair-fed 0.75 g/day of gum arabic added to 5 g of basal diet, the digestibility of gum arabic was 71% (5).

The caloric value of gum arabic was investigated in rats by supplementing their daily diets with various levels of gum arabic and observing its effect on growth rate. At dietary levels less than 5%, gum arabic exhibited a caloric value equivalent to that of sucrose, approximately 4 cal/g. As the dietary level of gum arabic was increased, the apparent caloric value decreased (3 g at 10%). At the 17% level, gum arabic produced no increase in growth beyond that observed in the control, indicating no caloric contribution. As the gum arabic level was increased further, the rate of growth progressively diminished, to the extent of being severely depressed at the 45% level. The apparent decrease in caloric value with increase in dose was explained as the result of inhibitory aspects of gum arabic. Digestibility data indicated that up to 80% of the gum arabic was absorbed (20, 21).

An average caloric value of gum arabic over a range of dietary levels (0.5 g, 1.0 g, and 1.5 g) in the rat, was reported as 2.48 cal/g (23).

Gum arabic was found to be highly digestible (90%) when administered to guinea pigs in their diet at a level of 15% for ten days (16).

III. Metabolism and Excretion

According to Monke (16), gum arabic administered orally to rats is excreted unchanged in the feces. However, studies by Booth (5), Shue (20, 21), and O'Dell (18) indicate that gum arabic is 70-90% utilized in the animal. The fate of the undigested gum was not determined by these workers. (See Biochemical Aspects - II)

Gum arabic injected intravenously in man is rapidly excreted in the urine (3).

IV. Effects on Enzymes and Other Biochemical Parameters

It has been demonstrated that gum arabic has the ability to inhibit the action of certain esterases (9).

There are a number of reported cases in which exposure to gum arabic has caused sensitization in man (3, 11, 12).

V. Drug Interaction

No information in sources obtained.

VI. Consumer Exposure Information

The following tables (I, III, IV and V) are preliminary reports on the use of gum arabic in regular foods (17). Table I shows the means of usage levels both usual and maximum. Table III shows the daily food consumption calculations for the food categories in which acacia is used. Table IV (Eaters Only) shows the daily intakes of Acacia when food in each indicated food category is eaten one or more times during any 14-day period. Table V (Total Sample) shows the intakes when food in each indicated food category may, or may not be eaten during any 14-day period. The intakes in these tables were calculated by multiplying the usage levels (Table I) by the food consumption data (Table III) and by a factor of 10 (to convert % to decimal values and grams to mg., or $(1/100) \times (1000 \text{ mg./gram}) = 10$). For each substance, the

daily intakes were calculated at four different levels by using four different combinations of usage level data (Table I) and food consumption data (Table III): (1) Average Intake = usual level of use x mean food consumption; (2) Normal High Intake A = usual level of use x high food consumption; (3) Normal High Intake B = maximum level of use x mean food consumption; (4) Very High Intake = maximum level of use x high food consumption. The designation "Eaters Only" means that the food consumption calculations per individual food category (and from these the daily intakes) were based only on those persons in the total MRCA sample of 12,743 persons who ate food in the specific food category one or more times during the 14-day MRCA survey period.

Table I--Preliminary Calculations on the Use of Acacia in
Regular Foods per Food Category†

No.	Food Category Name	Mean Usage Levels			
		(No. of Firms Reporting)	Usual Level, %	(No. of Firms Reporting)	Max. Level, %
01	Baked Goods	(30)	0.09915	(29)	0.21211
02	Breakfast Cereals	(4)	0.00693	(4)	0.02033
03	Other Grain Products	(*)	1.00000	(*)	1.00000
04	Fats and Oils	(*)	0.30945	(*)	0.81050
05	Milk Products	(*)	0.00400	(*)	0.00400
07	Frozen Dairy Products	(24)	0.03073	(22)	0.06575
08	Processed Fruit, Juices	(8)	0.09320	(8)	0.15939
09	Fruit and Water Ices	(*)	0.35633	(*)	0.71267
10	Processed Meat Products	(5)	0.05159	(5)	0.10392
15	Condiments and Relishes	(4)	0.00623	(4)	0.00693
16	Soft Candy	(27)	5.01499	(26)	6.41070
17	Confections, Frostings	(4)	3.36625	(4)	3.43325
19	Sweet Sauces, Syrups	(*)	0.03000	(*)	0.03000
20	Gelatin, Puddings	(24)	0.07492	(23)	0.26863
21	Soups, Soup Mixes	(*)	0.00400	(*)	0.00400
22	Snack Foods	(*)	0.40050	(*)	2.00060
23	Beverages--Type I	(55)	0.03386	(55)	0.07749
24	Beverages--Type II	(23)	0.02133	(22)	0.03881
25	Nuts, Nut Products	(4)	1.97000	(4)	2.19167
28	Dairy Products Analogs	(*)	0.95640	(*)	1.25000
30	Hard Candy	(3)	7.84803	(3)	13.25867
31	Chewing Gum	(9)	0.64470	(9)	1.51256
33	Sugar Substitutes	(*)	1.00000	(*)	1.00000
34	Instant Coffee and Tea	(*)	0.01333	(*)	0.08000
49	Unclassified, Misc.	(*)	0.60633	(*)	1.79848

†These calculations were made on the basis of usage level data submitted by 45 NAS firms, 60 FENIA firms, and 21 subsurvey firms. Many firms participated in more than one survey; therefore, the number of firms cannot be added to obtain an unduplicated total of firms reporting. No reports of any kind were received on the use of Acacia in baby foods.

*The asterisk in parentheses indicates that reports were received from only 1 or 2 firms.

Table III--Preliminary Daily Food Consumption Calculations per Food Category*

No.	Food Category Name	Daily Food Consumption, grams			
		Eaters Only		Total Sample	
		Mean	High	Mean	High
01	Baked Goods	137.3	203.9	137.2	203.8
02	Breakfast Cereals	26.8	55.5	20.0	51.8
03	Other Grain Products	34.2	67.3	27.8	61.4
04	Fats and Oils	17.8	31.8	17.5	31.6
05	Milk Products	56.8	155.4	39.5	120.8
07	Frozen Dairy Products	33.7	68.6	25.6	61.7
08	Processed Fruit, Juices	128.8	256.9	118.3	250.6
09	Fruit and Water Ices	11.5	23.9	0.7	2.5
10	Processed Meat Products	79.2	130.4	78.4	130.1
14	Processed Vegetables	85.5	143.4	85.0	143.2
15	Condiments and Relishes	10.9	22.9	8.8	21.2
16	Soft Candy	11.4	24.7	5.8	17.6
17	Confections, Frostings	3.4	6.2	0.3	0.8
18	Jams, Jellies, Preserves	11.0	25.2	5.7	17.7
19	Sweet Sauces, Syrups	12.3	24.4	6.8	17.9
20	Gelatin, Puddings	30.9	62.6	20.4	52.5
21	Soups, Soup Mixes	50.6	106.7	31.7	84.9
22	Snack Foods	2.1	4.6	1.3	3.7
23	Beverages--Type I	142.3	318.9	104.0	277.7
24	Beverages--Type II	204.1	504.0	28.0	58.3
25	Nuts, Nut Products	10.9	23.7	5.2	15.5
27	Gravies and Sauces	13.3	26.4	8.3	21.3
28	Dairy Products Analogs	5.8	17.0	0.9	1.5
30	Hard Candy	4.9	10.2	0.6	1.7
31	Chewing Gum	0.7	1.5	0.2	0.4
33	Sugar Substitutes	0.7	1.7	0.1	0.1
34	Instant Coffee and Tea	143.1	266.2	121.1	259.4

*These calculations are based on frequency-of-eating data obtained from the Market Research Corporation of America, and on portion size data obtained from the U.S. Department of Agriculture Spring 1965 Food Intake Survey. "Eaters Only" refers to that portion of individuals in the MRCA sample who ate foods in these food categories 1 or more times during the 14-day MRCA survey period. "Total Sample" refers to all 12,473 individuals in the MRCA survey, many of whom did not eat food in some of the food categories during the 14-day survey period.

Table IV--Preliminary Calculations on the Daily Intake (mg.)
of Acetate per Individual Food Category* (Eaters Only)

No.	Food Category	Average Intake ¹	Normal High Intake		Very High Intake
			Usual Use/ High Food ²	Max. Use/ Mean Food ³	Max. Use/ High Food ⁴
01	Baked Goods	136.13	202.17	291.23	432.49
02	Breakfast Cereals	1.86	3.85	5.45	11.28
03	Other Grain Products	342.00	673.00	342.00	673.00
04	Fats and Oils	55.08	98.40	144.27	257.74
05	Milk Products	2.27	6.22	2.27	6.22
07	Frozen Dairy Products	10.36	21.03	22.16	45.10
08	Processed Fruit, Juices	120.04	239.43	205.23	409.47
09	Fruit and Water Ices	40.98	85.16	81.96	170.33
10	Processed Meat Products	40.86	67.27	82.30	135.51
15	Condiments and Relishes	0.68	1.43	0.76	1.59
16	Soft Candy	571.71	1233.70	730.62	1503.44
17	Confections, Frostings	114.45	208.71	116.73	212.86
19	Sweet Sauces, Syrups	3.69	7.32	3.69	7.32
20	Gelatin, Puddings	23.15	46.90	83.01	168.16
21	Soups, Soup Mixes	2.02	4.27	2.02	4.27
22	Snack Foods	8.41	18.42	42.01	92.03
23	Beverages--Type I	48.18	107.98	110.27	247.12
24	Beverages--Type II	43.53	107.50	79.21	195.60
25	Nuts, Nut Products	214.73	466.89	238.69	519.43
28	Dairy Products Analogs	55.47	162.59	72.50	212.50
30	Hard Candy	384.55	800.50	649.68	1352.38
31	Chewing Gum	4.51	9.67	10.59	22.69
33	Sugar Substitutes	7.00	17.00	7.00	17.00
34	Instant Coffee and Tea	19.08	35.48	114.48	212.96

*Intakes are for adults and children (males & females), ages 2 to 65+ years. Calculations are based on food consumption data (Table III, Eaters Only) for individuals who eat food in these food categories 1 or more times during any 14-day period. Caution: The intakes for the 24 food categories in this table cannot be used to arrive at a combined total daily intake for these substances; for this information, see Table V. All calculations are based on the assumption that the substance is added to all foods in each category at the levels indicated (either usual or maximum, as in Table I).

¹Usual Level, $\%$ (Table I) \times Mean Food Consumption (Eaters Only, Table III) \times Factor

²Usual Level, $\%$ (Table I) \times High Food Consumption (Eaters Only, Table III) \times Factor

³Maximum Level, $\%$ (Table I) \times Mean Food Consumption (Eaters Only, Table III) \times Factor

⁴Maximum Level, $\%$ (Table I) \times High Food Consumption (Eaters Only, Table III) \times Factor

Note: The Factor is 10, which is necessary to convert $\%$ (usage levels) into decimal values, and to convert grams (food consumption) into mg. (intake of substance), or $(1/100) \times (1000 \text{ mg./gram}) = 10$.

Table V--Preliminary Calculations on the Daily Intake (mg.)
of Aroclor per Food Category and the Total Dietary* (TOTAL SAMPLE)

No.	Food Category Name	Average Intake ¹	Normal High Intake		Very High Intake
			Usual Use/ High Food ²	Max. Use/ Mean Food ³	Max. Use/ High Food ⁴
01	Baked Goods	136.03	202.07	291.02	432.26
02	Breakfast Cereals	1.39	3.59	4.07	10.53
03	Other Grain Products	278.00	614.00	278.00	614.00
04	Fats and Oils	54.15	97.79	141.84	256.12
05	Milk Products	1.58	4.83	1.58	4.83
07	Frozen Dairy Products	7.87	18.96	16.83	40.57
08	Processed Fruit, Juices	110.26	233.56	188.56	399.43
09	Fruit and Water Ices	2.49	8.91	4.99	17.82
10	Processed Meat Products	40.45	67.12	61.47	135.20
15	Condiments and Relishes	0.55	1.32	0.61	1.47
16	Soft Candy	290.87	882.64	371.82	1128.28
17	Confections, Frostings	10.10	26.93	10.30	27.47
19	Sweet Sauces, Syrups	2.04	5.37	2.04	5.37
20	Gelatin, Puddings	15.28	39.33	54.80	141.03
21	Soups, Soup Mixes	1.27	3.40	1.27	3.40
22	Snack Foods	5.21	14.82	26.01	74.02
23	Beverages--Type I	35.21	94.03	80.59	215.19
24	Beverages--Type II	5.97	12.44	10.87	22.63
25	Nuts, Nut Products	102.44	305.35	113.97	339.71
28	Dairy Products Analogs	8.61	14.35	11.25	18.75
30	Hard Candy	47.09	133.42	79.55	225.40
31	Chewing Gum	1.29	2.58	3.03	6.05
33	Sugar Substitutes	1.00	1.00	1.00	1.00
34	Instant Coffee and Tea	16.14	34.58	96.88	207.52
Totals (mg.) for all categories		1175.29	2822.39	1872.35	4328.07

*Intakes are for adults and children (males + females), ages 2 to 65+ years. Calculations are based on food consumption data (Table III, Total Sample) for individuals who may or may not eat food in these food categories 1 or more times during any 14-day period. The intakes for the 24 food categories may be (and have been) added to arrive at a combined total daily intake for the substance. All calculations are based on the assumption that the substance is added to all foods in each category at the levels indicated (either usual or maximum, as in Table I).

¹Usual Level, % (Table I) x Mean Food Consumption (Total Sample, Table III) x Factor

²Usual Level, % (Table I) x High Food Consumption (Total Sample, Table III) x Factor

³Maximum Level, % (Table I) x Mean Food Consumption (Total Sample, Table III) x Factor

⁴Maximum Level, % (Table I) x High Food Consumption (Total Sample, Table III) x Factor

Note: The Factor is 10, which is necessary to convert % (usage levels) into decimal values, and to convert grams (food consumption) into mg. (intake of substance), or (1/100) x (1000 mg./gram) = 10.

GUM ARABIC

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SENSITIVITY TO GUM ACACIA, WITH A REPORT OF TEN CASES OF ASTHMA IN PRINTERS

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ISOLATED reports of sensitivity to acacia (gum arabic) have appeared in medical literature. We wish to review these, to discuss the chemistry and physiologic action of acacia, and to report ten cases of asthma caused in printers by this material.

Spielman and Baldwin¹ in 1933 presented a case they believed to be the first of proved sensitivity to gum arabic. The patient worked in a candy factory. Levin² reported one case in a printer, and Marsh³ has recently reported three cases of acacia sensitization that he observed, all in printers. Maytum and Magath⁴ reported nasal obstruction, lacrimation, difficulty in breathing, cough, loss of voice, and a suggestion of laryngeal stridor in a 27-year-old girl a few minutes after the injection of this gum. These observers considered the reaction anaphylactoid. They emphasized that it was the first allergic reaction in over 3,000 acacia infusions given at the Mayo Clinic. It is interesting to note that the patient had received a similar infusion seven months earlier. The authors comment that acacia is given for shock and a second infusion to the same patient is quite rare.

Studdiford⁵ has reported severe and fatal reactions following intravenous use of acacia-glucose infusions. In all of his patients, the reaction consisted of cyanosis, dyspnea, tachycardia, edema of the lungs, and a sense of impending death. Several of these reactions occurred shortly after obtaining a new pharmaceutical supply of the drug. The author was particularly impressed with the autopsy findings of liver damage. He also attributed part of the reaction to poor absorption of gases caused by the gum which coated the red blood cells, as Christie and co-workers⁶ have demonstrated. It has been the opinion of some workers that the reactions were caused by a poorly prepared acacia solution.^{5, 7}

Chemically, acacia is classed as an inert colloid. It is considered to be a polysaccharide member of the carbohydrate family, related polysaccharides being glycogen, dextrin, cellulose, and starch. All are amorphous, odorless, and translucent, and on hydrolysis yield one or more sugars, usually pentoses and hexoses.

Uhlenhuth and Remy⁸ found 0.3 per cent of nitrogen present in samples of purified gum acacia. Spielman and Baldwin¹ found 0.5 per cent of total nitrogen in an analysis of this material and noted a positive biuret test indicating the presence of protein nitrogen. In an

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analysis of three samples purchased on the open market, we have found an average of 0.48 per cent of nitrogen and a positive biuret test.

Uhlenhuth and Remy⁸ have demonstrated the presence of precipitins and complement-fixing antibodies in rabbits injected with purified acacia. No such reactions were produced when starch, dextrin, gelatin, tragacanth, gum cherry, or gum gutta was used.

Maytum and Magath⁴ injected nineteen guinea pigs intraperitoneally with acacia and four weeks later gave them an intravenous injection of the same material. In seven there was no anaphylactoid reaction; in four the reaction was mild; in three, fairly severe; in two, severe; and in three, fatal. They concluded that this gum had antigenic properties.

Spielman and Baldwin¹ tested thirty routine patients for gum arabic sensitivity by scratch and intradermal methods and found no positive reactions. These authors quote the findings of Farrar in patients receiving intravenous infusion of acacia, with negative skin tests ten to twenty-five days after infusion.

Acacia has many uses, among which are the following:

Commercial

Shoe polish manufacture
Water colors, transparent
Metal polish manufacture
Various paints
Many varnishes
Linoleum
Oil cloth
Match heads
Printing textiles
Porcelain
Ceramics
Spray for paper sizing
Textile fibers

Fireworks
Mucilage
Glues
Cements
Adhesive pastes
Confections
Sweetmeats
Bakery products
Process engraving
Potteries
Stiffening
Luster for crepe silk
For body and drier in writing
and lithograph inks

Medical

Mucilage of acacia
Vehicle and pill excipient
Emulsifier

The cases of asthma studied were in printers who were sensitive to the acacia used in "offset spray." This spray is being employed more frequently because of the use of colored ink in an increasing number of publications. As each sheet is printed, the spray comes in contact with it, and this forms a fine film over the freshly colored sheet, thus preventing "offset" or smearing of the colored matter onto the next sheet added to the stack. With the spray machines used for this work, a coating of the solution has been noted on workmen's glasses 20 feet from the machine. With proper lighting, one may see a mist spreading well beyond the printing press.

CASE REPORTS

CASE 1.—P. A., white male, married, aged 53 years, a printer for eleven years, was first seen on Feb. 19, 1938, at which time he complained of asthma and severe coughing attacks. He had developed influenza in December, 1937; this was fol-

lowed by some tightness in his chest. He had no fever or cough. He was able to continue his work, but he gradually developed breathlessness during the next three months and had two severe attacks of asthma which were relieved by the administration of epinephrine hydrochloride. He had noticed that he was more dyspneic while at work. He had no other allergic manifestations, and there was no family history of allergic diseases. Except for an attack of lumbago six or eight years before, he had always been in good health.

This patient was a well-developed, well-nourished, adult male. The blood pressure was 122/80; the pulse rate varied from 86 to 90, with occasional extrasystoles. The heart sounds were normal, and there was no cardiac enlargement. The chest was emphysematous, with dry asthmatic râles audible throughout the lung fields. The remainder of the physical examination was negative.

Cutaneous tests with 150 inhalant, pollen, and food allergens were negative. Nasal smears were negative, as were blood and urine examinations. Stained sputum smears showed from 16 per cent to 24 per cent eosinophilic staining cells. Stereoscopic roentgenograms of the chest showed a normal-sized heart with a thickening of the basilar trunk markings. X-ray examination of the accessory nasal sinuses revealed evidence of chronic infection.

The patient was again seen on Sept. 26, 1938, at the Long Hospital of the Indiana University Medical Center. During the intervening seven months the usual measures had been carried out to determine the cause of his asthma, and various drugs, including digitalis, had been given. There had been no lessening of symptoms. Physical examination on this admission was essentially the same as previously observed except for a low-grade paranasal sinusitis and 18 pounds weight loss.

Repeat cutaneous and intracutaneous allergic tests were negative except for a four-plus reaction to gum arabic. (A second printer with asthma had been found sensitive to offset spray in March, 1938.) The history then revealed that an "offset spray" had been installed at his place of work in June, 1937, six months before the onset of his asthma.

The patient was advised to change his occupation. He refused to comply with this request and continued to show symptoms until a dextrose base solution was substituted for the acacia material in the offset spray. Subsequent examinations revealed freedom from symptoms.

CASE 2.—H. D., white male, aged 29 years, was admitted to the University of Michigan Hospital on May 27, 1938, complaining of difficulty in breathing of three months' duration. In February, 1938, two weeks after beginning work in a printing establishment, he noted difficulty in breathing, associated with wheezing. His work was that of spraying a "drying material" on printed matter in the plant. Several other workers had some "cough and congestion in the morning," but he knew of no cases with complaints as severe as his own. There was no history of previous allergic manifestations. One sister suffered with hay fever.

The physical examination on his first visit was not contributory. Four days later he was seen again, and examination showed the skin to be warm and flushed and the temperature to be 99° F. There were sibilant and sonorous râles over the lung fields.

Examination of the blood showed no eosinophilia. A nasal smear was likewise negative. Stereoscopic x-ray examinations of the chest showed no definite abnormalities. Cutaneous and intracutaneous testing disclosed moderate reactions to June grass, orchard grass, timothy, and short ragweed, rather marked reactions to stock house dust, and a good reaction to gum acacia.

In view of the history which suggested that the spray might be a possible cause of his symptoms, a cutaneous test was made with "drying spray," with resultant marked reaction. On June 14, 1938, he was placed in a small

which was then sprayed with a solution of alcohol, ether, and gum acacia. Following a fifteen-minute exposure in the room he developed typical allergic conjunctivitis and rhinorrhea and a sensation of nasal obstruction with objective evidence of an allergic rhinitis. The following day the above procedure was repeated, with development of typical allergic conjunctivitis, allergic rhinitis, and mild asthma. The patient's symptoms promptly disappeared upon change of occupation.

Eight other cases of sensitivity to acacia have been observed. All patients worked in printing plants. Two of them were women.

Acacia seems to be a highly specific allergen. All ten patients gave strongly positive direct tests to this material, and the sera of three gave positive transfer tests. Passive transfer tests were not made on the other seven patients. The direct transfer tests were negative to Indian gum and tragacanth. Sixteen highly allergic patients and ten normal controls all gave negative reactions to gum arabic. One of us (C. B. B.) has tested routinely with it for the past eighteen months. Results have been negative.

SUMMARY

1. A group of ten patients in whom direct exposure to acacia initiated severe asthmatic seizures have been studied. All were exposed to this material by offset sprays used in the printing industry.
2. Apparently acacia (gum arabic) is an industrial hazard, and sensitization to it is not rare. It is suggested that dextrose be substituted for acacia in offset sprays.
3. The time of gross exposure to this spray material before the onset of asthmatic symptoms varied from two weeks to twelve months.
4. Eight of the ten patients were adult males ranging in age from 22 to 53 years. This high incidence of males may be explained by the fact that relatively few females are employed in the printing industry.
5. The increasing use of gum arabic in the printing of magazines, box labeling, and other industrial sources presents the possibility of a widespread allergy to acacia.

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DISCUSSION

DR. SAMUEL H. HURWITZ, San Francisco.—In 1916, Dr. George H. Whipple carried out a series of experiments, in which I was associated, in an attempt to produce marked secondary hemorrhages. These studies led to the discovery of the antianemic substances in liver. We found that by plasmapheresis, that is, the removal of the red corpuscles and the replacement of the blood volume with a mixture of 6 per cent acacia (gum arabic) in saline solution, we could preserve the life of those animals for a longer period of time without having them die as a result of tremendous drop in the blood pressure. We did not understand the importance of the gum as a potent factor in the production of acute anaphylactic shock at that time. But some of the animals, after an incubation period and a subsequent infusion of acacia in saline, died in acute anaphylactic shock. The pathologic studies carried out by Dr. Whipple showed the typical classical lesions of anaphylactic shock in dogs. There were lesions in the liver, chiefly necrosis, and also hemorrhagic edema of the mucous membranes high in the small intestine.

Despite supplemental studies, some of us used infusions of acacia in saline in man in case of surgical shock at the time, because twenty-four years ago we did not have available the ready method of transfusion and the preserved bloods which we have at the present time. Despite the use of the infusions of acacia in saline in man in surgical shock, which were subsequently used very extensively in the World War, we heard very little about the clinical effects of infusions of acacia in saline. About 1922, we began to see reports in the literature concerning its untoward effects. But despite the thousands of cases in which such infusions have been used, we heard very little about the allergic manifestations to which Dr. Bohner called our attention.

Of course, it is very easy to understand. In the first place, as Dr. Bohner stated, these patients received one infusion of saline and acacia, rarely received the second, and, if they did receive the second, it was at a considerable interval after the first. Probably also because of the rapid elimination of the acacia in the urine, we may not have seen these marked clinical manifestations of allergy following infusions of acacia in saline.

The method of absorption in the spray, however, is quite a different thing. There one is dealing with repeated exposures and slow absorption of acacia by the membranes of human beings.

DR. SHELDON.—We do not wish to enter any argument for or against the use of acacia infusions, but we do wish to emphasize the possibility of severe allergic reactions occurring from indiscriminate use of acacia for intravenous infusions.

THE IMPORTANCE OF BULK IN THE NUTRITION OF THE GUINEA PIG¹

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In spite of the rather extensive studies on the nutritional requirements of the guinea pig (cavy) it still is not possible to devise a purified ration that will produce "normal" rates of growth. Cannon et al. ('45) reported that growth of less than 7 to 8 gm per day from the second to the 8th weeks of life cannot be considered "normal" if growth on natural foodstuffs is accepted as the standard. These same workers concluded that growth and survival on purified rations, supplemented with linseed oil meal and solubilized liver, was poorer than on a crude diet and that the animals fed the former developed anemia and leucopenia. Woolley and Sprince ('45) described three guinea pig factors (abbreviated, G.P.F.) numbered 1, 2, and 3. They stated that G.P.F.-1 was folic acid, G.P.F.-2 was replaceable by a mixture of cellulose and protein, and G.P.F.-3 was an as yet unidentified factor present in solubilized liver extracts. However, the best growth obtained when all three of these factors were added to a purified basal ration was only 4 gm per day during a period of 4 weeks.

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The guinea pig, unlike the rat, is an herbivorous animal, subsisting entirely on foods of plant origin. Therefore, it is not surprising to find that the digestive tract is different, in that it includes a large functioning caecum constituting about 15% of the body weight. The results of the present investigation will be cited in an attempt to relate this peculiarity in the lower intestinal tract to the importance of bulk in a purified ration.

EXPERIMENTAL

There is abundant evidence to indicate that the guinea pig is very susceptible to certain diseases, especially *Salmonella* infections and pneumonia. For this reason two precautions were taken: first, the animals were obtained from a single source during the entire investigation and, secondly, the animals were housed in a room of uniform temperature (75 to 85°F.), in individual wire bottom cages which were steam sterilized before being used. Water was supplied by means of bottles hung on the outside of each cage with a glass tube and drinking tip on the inside of the cage.

A commercial ration² was used as the positive control diet. It is known to be high in alfalfa leaf meal and has always resulted in excellent growth when used for our animals. The basal synthetic diet had the following composition: Sucrose, 60; casein³ (vitamin free), 30; salts IV, 4; fortified soybean oil, 4; sucrose mixture containing B vitamins, 0.8; and choline, 0.3 parts. The fortified soybean oil was prepared by dissolving β -carotene in chloroform and distributing it as a layer on the sides of a round-bottom flask while removing all of the chloroform with reduced pressure. Soybean oil was added to the flask and heated (60-70°) on a sand bath for three or 4 hours until the carotene was dissolved in the oil. The solution was allowed to cool and crystalline vitamin D₂ and vitamin K as menadione were added. Finally alpha-tocopherol was added and the fortified oil made up to a final weight. The use

² Rockland guinea pig pellets.

³ Obtained from General Biochemicals, Inc., Chagrin Falls, Ohio.

of 4% of this oil supplied 1.2 mg of β -carotene, 12 mg of α -tocopherol, 8 mg of calciferol, and 0.2 mg of vitamin K per 100 gm of ration. The added mixture of B vitamins supplied 1 mg of thiamine hydrochloride, 1.4 mg of riboflavin, 1 mg pyridoxine, 3 mg calcium pantothenate, 200 mg inositol, 10 mg niacin, 10 mg *p*-aminobenzoic acid, 0.04 mg biotin, and 0.3 mg folic acid per 100 gm of ration. Vitamin C was supplied by dissolving sufficient ascorbic acid in a 70% sugar solution to secure 100 mg per milliliter. This solution was fed with individual pipettes at a level of 0.25 ml every other day, which was equivalent to 12.5 mg per animal per day.

Loss of guinea pigs during the first few weeks on experiment was completely eliminated in most groups of animals if small amounts of the stock ration were placed on top of the experimental diet during the first 7 days. Approximately 4 or 5 gm were used the first day and the amount decreased each day until no addition was made after the 7th day. In spite of these precautions, a high mortality was observed in a few of the experimental groups. No explanation could be found for these results, and when the condition was encountered the entire group of animals was discarded. It should be emphasized that animals from these groups never failed when fed the stock ration.

RESULTS

The growth data are given in table 1. Since the rate of growth of the animals in different groups varied to some extent, the results are separated into several series. When the commercial stock ration, supplemented with vitamin C, was fed, an average daily gain in weight of 6.9 gm was obtained over a 6-week period. The basal synthetic ration, on the other hand, which contained all of the known dietary factors except vitamin B₁₂, gave a growth response of only 1.8 gm per day. When crude casein was used in place of the vitamin-free casein the same results were obtained. No improvement resulted when dextrin was used in place of sucrose. Since one of the major components of the stock ration was alfalfa leaf

TABLE 1
Growth of guinea pigs on different rations¹

	NO. OF ANIMALS	WEEKS ON EXP.	AVERAGE GAIN IN WT. gm/day
<i>Series I</i>			
Rockland stock diet (Control diet)	30	6	6.9
Basal synthetic	9	6	1.8
Basal synthetic (crude casein)	7	6	1.9
Basal synthetic (Cetrin)	3	6	1.9
Basal synthetic + 15% alfalfa leaf meal	4	6	3.6
Basal synthetic + 25% alfalfa leaf meal	4	8	<u>6.5</u>
Basal synthetic + 25% autoclaved alfalfa leaf meal	5	6	<u>6.5</u>
Basal synthetic + 30% beet pulp	4	6	<u>6.9</u>
Basal synthetic + 10% beet pulp fiber	4	6	2.0
Basal synthetic + 20% beet pulp fiber	2	6	3.1
Basal synthetic + 20% pectin	3	6	4.4
1. Basal synthetic + 20% gum arabic	3	6	5.2
Basal synthetic + 15% cellul flour	9	6	4.6
<i>Series II</i>			
2. Basal synthetic + 15% gum arabic	20	6	5.1
Basal synthetic + 15% xylose	3	3	3.5
Basal synthetic + 2% levulinic acid	4	4	2.5
Basal synthetic + hydrolyzed gum arabic \approx 15%	5	4	2.5
<i>Series III</i>			
3. Basal synthetic + 15% gum arabic + summer milk ad lib.	3	6	4.7
4. Basal synthetic + 15% gum arabic + extract of beef liver \approx 10%	5	6	5.7
5. Basal synthetic + 15% gum arabic + 15% brewers' yeast	5	6	<u>6.7</u>
6. Basal synthetic + 15% gum arabic + 4% fish solubles	6	6	2.5
7. Basal synthetic + 15% gum arabic + 6% grass juice powder	6	6	4.6
8. Basal synthetic + 15% gum arabic + 4% grass juice	6	6	2.8

TABLE 1 (continued)

	NO. OF ANIMALS	WEEKS ON EXP.	AVERAGE GAIN IN WT. gm/day
<i>Series IV</i>			
Rockland stock diet (Control diet)	4	9	6.6
9. Basal synthetic + 15% gum arabic	5	9	5.1
10. Basal synthetic + 15% gum arabic + alfalfa ash \approx 25%	5	9	5.4
11. Basal synthetic (crude casein) + 15% gum arabic	5	9	5.5
12. Basal synthetic (crude casein) + 15% gum arabic + alfalfa ash \approx 25%	6	9	7.0
13. Basal synthetic (crude casein) + 15% gum arabic + stock ration ash \approx 100%	4	9	5.7
Basal synthetic + alfalfa ash \approx 25%	5	9	4.4
<i>Series V</i>			
14. Basal synthetic + 15% gum arabic	10	6	5.1
15. Basal synthetic (20% casein) + 15% gum arabic	10	6	2.3
16. Basal synthetic (20% crude casein) + 15% gum arabic	9	3	2.4
17. Basal synthetic (20% casein) + 15% gum arabic + 0.3% L-cystine + 0.1 DL-tryptophan	5	6	4.1
18. Basal synthetic (20% casein) + 15% gum arabic + 1% DL-arginine + 0.1% L-cystine + 0.2% glycine	2	6	2.5
19. Basal synthetic (15% casein) + 15% gum arabic + 15% wheat gluten	3	6	4.7
20. Basal synthetic (8% casein) + 15% gum arabic + 8% fibrin + 8% egg albumin	3	6	4.2
21. Basal synthetic (20% casein) + 15% gum arabic + 7% protolysate	4	3	2.7
22. Basal synthetic (20% casein) + 15% gum arabic + extract of crude casein \approx 50%	6	3	3.0
23. Basal synthetic (30% special casein) + 15% gum arabic	5	4	5.1
Basal synthetic (18% casein) + 30% beet pulp	4	6	5.1
Basal synthetic (28% casein) + 30% beet pulp	4	6	5.2

¹ All additions made at the expense of the sucrose in the basal ration.

meal, experiments were set up to determine if the addition of this material would improve the growth of the animals. When 25% of leaf meal was added to the basal synthetic ration the average rate of growth was equal to that obtained on the stock ration, but 15% of the meal was definitely inadequate. Apparently alfalfa supplied all of the missing dietary factors when added at the higher level. The effect of the alfalfa was not altered when the meal was autoclaved.

Woolley and Sprince ('45) recognized dietary bulk as an important factor in the growth of guinea pigs and used cellul flour and cellophane to satisfy this requirement. In order to study the effect of bulk more extensively, it was decided to try beet pulp, a material which contains more than 20% crude fiber. Plain dried beet pulp was added to the ration at a level of 30%. When this addition was made the gain in weight over a 6-week period was equal to or better than that obtained with 25% alfalfa leaf meal. However, when the crude fiber from beet pulp was isolated and fed at levels of 10 and 20%, poor growth resulted. These results were somewhat unexpected and led to an examination of the chemical analysis of beet pulp. It was found that beet pulp contains as much as 30% of pectin, which is a hemicellulose (Codling and Woodman, '29).

The following materials were therefore tested in order to differentiate between the effects of cellulose and hemicellulose: Cellu flour, cellophane, pectin,⁴ gum arabic, gum mesquite, gum tragacanth, oat straw, agar, cornstarch, potato starch, paper pulp, wood shavings, silica gel and methyl cellulose. Of all of these materials, powdered gum arabic consistently produced the best response. Pectin, agar, oat straw, cellu flour and cellophane stimulated growth to some extent but to a lesser degree than gum arabic. The other materials were either inactive or only slightly active.

Many of the animals receiving the basal ration plus gum arabic were continued on the ration after the 6-week period.

⁴Obtained through the courtesy of the California Fruit Growers Exchange, Ontario, California.

and these animals continued to grow and showed no indication of a serious deficiency during a period of 8 months or more. Post-mortem examination of guinea pigs failing to survive on the basal ration without gum arabic revealed no specific abnormalities. One symptom which was noted was that the animals ate the hair on the abdomen and sides of the body. This was observed in animals on almost all the inadequate rations but never in more than two out of 5 covies on the same ration. Another symptom which was frequently observed on adequate as well as inadequate diets was diarrhea. The duration and severity of the condition varied greatly. Occasionally rapidly growing animals would suddenly lose as much as 50 gm in body weight, at which time a severe diarrhea developed. However, all of the animals on any given ration did not exhibit such tendencies. The feces from animals receiving the stock rations were always hard and dry in appearance, whereas the feces from guinea pigs on purified rations were unusually soft, even when the rate of growth was normal.

When it was found that gum arabic was active in promoting growth there were at least two questions to be answered; first, how does the gum function and, secondly, are there any other missing dietary factors to be contended with when a ration containing 15% gum arabic is fed? Gum arabic contains 28.3% galactosoglucuronic acid, 29.5% galactose, 34.4% arabinose and 14.2% rhamnose hydrate. Arabinose was not available, but in order to test the activity of a pentose sugar, xylose² was fed at a level of 15%. Poor growth resulted (series II). Davis and Briggs ('47) reported that materials like Ruffex, wood shavings, and cellulose flour were capable of stimulating the growth rate of chicks when added to a purified ration at levels of 5 to 15%. These investigators also found that the degradation products of cellulose, furfural and levulinic acid, were equally active. Guinea pigs were fed a ration containing 2% levulinic acid but negative results were obtained. The most critical experiment designed to determine

² Obtained through the courtesy of the Northern Regional Research Laboratory, Peoria, Illinois.

whether the gum could be replaced by degradation products involved hydrolysis of gum arabic with dilute H_2SO_4 (Butler and Cretcher, '29), removal of the sulfate with $CaCO_3$, and then addition of the filtrate to the purified ration. Again the results, as judged by the average gain in weight, were subnormal and it was concluded that the intact gum molecule was essential for growth-promoting activity, probably due to a "bulk" effect on the lower intestinal tract.

The average growth rate for 20 animals receiving a ration containing 15% gum arabic was 5.1 gm per day (series II). Since a growth rate of 6.9 gm per day was obtained on the stock ration, it appeared that gum arabic was not the only factor involved. Various natural supplements were added to a ration containing 15% gum arabic, including raw summer milk (ad libitum) fish solubles, grass juice powder,² grass juice (20% solids),³ a liver extract and yeast (series III). Yeast produced a good response and the methanol extract of beef liver produced some response but the other supplements, namely milk, fish solubles, grass juice powder, and grass juice, appeared to depress rather than enhance growth.

Since 25% alfalfa leaf meal was capable of bringing about a normal growth rate, fractionation of this material was undertaken. It was found that an ether extract, a 90% ethanol extract, a methanol extract, or hot water extracts were all less active than the residues from which these extracts were prepared. The next logical fraction to test was the ash. Surprisingly enough, the ash of alfalfa leaf meal showed some effect when added to purified rations either with or without gum arabic (series IV). In fact, nearly normal growth rates were obtained when a ration containing 15% gum arabic plus alfalfa ash equivalent to 25% was fed. This ration has been tested several times and the results were nearly always in the normal range during the third to the 6th week of the experiment. The growth rate was often subnormal for the first three

² Obtained through the courtesy of the Cerophyl Laboratories, Kansas City, Missouri.

³ See footnote 5.

weeks of an experiment, after which nearly normal growth occurred. Whether this initial lag in growth is due to a missing dietary essential or simply a refusal on the part of the guinea pig to accept this ration readily is not known.

Stock ration ash as the only source of minerals in the ration (the ash from 100 gm of stock ration was substituted for 4 gm of salts IV) was as effective as alfalfa ash. The use of crude casein had some beneficial effect, especially in the presence of alfalfa ash. However, when the animals were continued on this ration for 12 weeks there was little difference between the animals receiving the purified and the crude casein.

Attempts have been made to determine what particular constituent in the alfalfa accounts for the activity observed. Cobalt, boron and molybdenum have been added singly and together without success. Sodium, magnesium, calcium and phosphorus have also been eliminated as being involved. The level of salt mixture in the basal ration (salts IV, Hegsted et al., '41) was raised from 4 to 8% with no improvement. The ash of alfalfa is quite basic and this may exert a favorable effect on some of the microflora of the lower tract by raising the pH. Another possibility which must be explored is the sodium-potassium ratio, because alfalfa ash contains a high level of potassium. Further work is in progress to clarify this question.

Van Wagtendonk and Wulzen ('43) observed the development of a wrist-stiffness in guinea pigs on a ration containing vitamin-fortified skim milk powder plus wheat straw. The deficiency could be corrected by feeding fresh cream or cane juice or crystalline material isolated from these sources. This wrist-stiffness has never been observed in the investigations reported here, and there was no response as measured by growth when either ergostanol⁸ (Oleson et al., '47) or the Wulzen anti-wrist stiffness factor⁹ was added to a purified ration. The

⁸ Obtained through the courtesy of the Lederle Laboratories, Pearl River, New York.

⁹ Obtained through the courtesy of the Lilly Research Laboratories, Indianapolis, Indiana.

possibility also arose that vitamin P active compounds such as hesperidin might be stimulatory for growth, but here again negative results were obtained when this substance was tried.

One other question was investigated, namely, the necessity for a high level of casein (30%) in the basal ration. If the level of casein was reduced from 30 to 20% in a ration containing 15% gum arabic, the growth was markedly reduced (series V). It will be recalled that Woolley and Sprince ('45) recognized this fact and they were able to secure growth equal to that obtained with 30% casein by adding arginine (1.0%), cystine (0.1%), and glycine (0.2%) to a ration containing 20% casein. The addition of several amino acids to the 20% casein ration did not improve growth (series V). The commercial stock ration contains only 18% crude protein and excellent growth is always obtained when it is fed. Why then should purified rations containing 20% casein fail? Kuiken et al. ('44) concluded that crude casein contained an unknown dietary factor essential for guinea pigs. Several other experiments were conducted including the addition to purified rations containing gum arabic of wheat gluten, a pancreatic digest of casein,¹⁰ fibrin, egg albumin, and laboratory-prepared cold acetic acid precipitated casein. None of these supplements was able to increase the growth rate to that obtained with 30% casein. Quite recently two rations were compared, both containing 30% beet pulp but one with 18 and the other with 28% crude casein. It was rather surprising to find that the growth rates at the end of 6 weeks were practically the same (5.1 and 5.2 gm per day). These rations also contained dextrin in place of sucrose and cellulose flour (3%). However, the fact that the ration containing 18% crude casein produced as good response as the one containing 28% suggests two possibilities: Either the protein (2%) supplied by the 30% beet pulp was an efficient supplement to the casein, or the lower level of protein is adequate when a proper source of bulk is present.

¹⁰ "Protolysate."

DISCUSSION

The results presented in this paper clearly demonstrate the beneficial effect of gum arabic when added to a purified diet for guinea pigs. The exact mechanism by which this gum functions is still unknown. It is known that the class of gums to which this one belongs are hydrophylic in nature and hence have considerable power to retain water and to give colloidal suspensions. Such a gum may function by affecting the type of bacteria multiplying in the lower part of the digestive tract. The gum may favor certain organisms which are able to synthesize unknown factors which are then made available to the host either by direct absorption, by coprophagy or by both mechanisms.

On the other hand, the hemicellulose may tend to decrease the number of unfavorable organisms. Diarrhea which might well owe its origin to an abnormal intestinal microflora, was frequently noted when guinea pigs were fed the unsupplemented rations. Furthermore, the addition of ash from alfalfa has a definite effect and this effect is more apparent in the presence of gum. Further studies are needed to determine which of the mineral elements may be concerned. Perhaps the most important factor is the proper balance among the ash elements that are present in predominant amounts. It is also interesting that the presence of hemicellulose material, especially in the form of beet pulp, tends to decrease the protein requirement. There is little reason to believe that the casein itself supplies a limiting factor; instead, the necessity for the higher level is probably related to the lack of bulk in the diet.

SUMMARY

A purified basal ration containing all of the known nutrients (except vitamin B₁₂) produced a rate of growth of only 1.8 gm per day for 6 weeks compared to a normal rate of 6.9 gm per day when a commercial ration composed of natural feeds was fed. When the purified basal ration was supplemented with 25% alfalfa leaf meal (in place of an equal amount of

current, a nearly normal average growth rate of 65 gm per day was obtained. When the basal ration was supplemented with 80% dried beet pulp growth was equally good. Powdered gum arabic (a hemicefalus), was consistently the most active single supplement when fed at a level of 15%. However, the average growth rate of 54 gm per day when this gum was fed was less than the normal rate of 65 gm. When the ash from alfalfa leaf meal equivalent to 25% was added to a ration containing 15% gum arabic, nearly normal growth rates were obtained for periods as long as 12 weeks. The level of protein in the synthetic ration could be reduced when gum arabic was fed.

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Physiologic Effects of Three Microbial Polysaccharides on Rats

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Recently two polysaccharides of unusual constitution and properties (Jeunes *et al.*, 1961b) have been produced under practical conditions (Anderson *et al.*, 1960; Rogovin *et al.*, 1961a,b) by microbial action on glucose. These biosynthetic, high molecular weight substances are water soluble and give stable, viscous solutions (Jeunes *et al.*, 1961a,b). Possible applications include their use as thickening and stabilizing agents for food and pharmaceutical products. Dextran, another high molecular weight polysaccharide, is produced biosynthetically from sucrose (Sohns *et al.*, 1954). The water-soluble dextran produces solutions of relatively low viscosity (Jeunes *et al.*, 1948). It has been shown to be a source of liver glycogen in rats (Bloom *et al.*, 1952). A partially degraded dextran for intravenous infusion has been the subject of metabolic study (Terry *et al.*, 1954).

Presented in this report are acute toxicity studies with mice and subacute toxicity studies with rats, of the recently produced polysaccharides. They include paired feeding tests and digestibility and caloric availability studies. For comparative purposes, dextran, gum guar, pectin, gum arabic, and agar were also included.

MATERIALS AND METHODS

Materials

Three exocellularly produced microbial polysaccharides were supplied by the Northern Regional Research Laboratory, Peoria, Illinois, as follows: phosphomannan Y-2448 (designated PM in this report) from the yeast *Hansenula holstii* NRRL Y-2448 (Anderson *et al.*, 1960; Rogovin *et al.*,

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1961a); polysaccharide B-1459 (designated PS) from the bacterium *Xanthomonas campestris* NRRL-1459 (Rogovin *et al.*, 1961b); and dextran B-512F from *Leuconostoc mesenteroides* NRRL B-512F (Sohns *et al.*, 1954). These products were isolated and purified in the form of dry powders of high purity and were free of viable cells. PM, the potassium salt of a diesterorthophosphomannan, is comprised of D-mannose units, orthophosphate, and potassium in the molar ratio of 5:1:1 (Jeunes *et al.*, 1961a). PS is comprised of D-glucose, D-mannose, and D-glucuronic acid (as the potassium salt) in the molar ratio of 3:2:2 and contains, in addition, about 5% O-acetyl groups (Jeunes *et al.*, 1961b). Dextran, a polymer of D-glucose, has 95% 1,6-linkages.

Other polysaccharides including low and high molecular weight dextrans, gum guar, agar, gum arabic, and pectin were purchased from commercial sources.

Basal diet A was a commercially formulated rat diet, to which the various polysaccharides were added at the expense of a portion of the entire diet.

Basal diet B was hand-mixed and consisted of the following ingredients (in per cent): corn meal 51.5, crude casein 11.5, linseed oil meal 10.0, alfalfa meal 2.0, cod-liver oil 3.0, bone ash 1.5, sodium chloride 0.5, and cornstarch 29.0. Test materials were fed at the expense of cornstarch.

In the initial tests, the constancy of the basal diet ingredients were maintained by substituting test materials at the expense of cornstarch only (basal diet B). Since the levels of test materials used were relatively low, substitution at the expense of the entire diet (basal diet A) was not considered objectionable, and this method was used in one test.

Methods

Acute toxicity was studied in mice by stomach tube, intraperitoneal, and intravenous administration. Albino rats were used in subacute toxicity and feeding tests and in digestibility and caloric availability studies. Skin irritation and skin sensitization were studied with rabbits and guinea pigs, respectively.

Weanling albino rats from our colony were separated into uniform groups and housed with wood shavings as bedding. They were allowed free access to water and the diets indicated.

Weekly records of food intake and body weight gains were maintained. Upon termination of the feeding period, all rats were autopsied, examined

grossly, and organ weights taken. Various tissues were preserved in 10% formalin, and blocked, stained, and sectioned for histopathologic examination.

The caloric availability and digestibility¹ of the polysaccharides were evaluated according to the procedure described by Rice *et al.* (1957). However, a modification was introduced which involved the determination of weight loss relative to the unsupplemented control group when all rats receiving supplements were switched to the basal diet only for a period of 48 hours, immediately after the 7-day supplementation period.

RESULTS

Acute Toxicity Studies

Adult mice received via stomach tube a total of 4 doses in water of 8 g PM per kilogram body weight over a period of 8 hours. Only 1 g PS per kilogram body weight could be given because of its greater viscosity. No effect of the treatments was observed. Intraperitoneal injections into mice were made daily for two 5-day periods separated by 2 days. Each injection consisted of 0.5 ml of water containing either 10 mg PM or 5 mg PS. Abdominal swelling was noted during the injection period, but during the treatment period and up to 2 weeks after the final injection, there were no deaths. The absence of PM or PS in the abdominal cavity at autopsy suggests that the injected material was absorbed. A dosage of 0.2 ml of 5% PM in 0.9% saline was injected into mice via tail vein without effects other than momentarily slightly irregular breathing.

Daily topical applications of a 5% aqueous solution of either PM or PS to shaved areas of rabbit skin caused localized irritation followed by skin cracking and bleeding. The application of water-soluble cornstarch solutions produced similar effects, probably due to continuous moistening of the skin. As the moisture evaporated, a hard film was deposited on the skin surface and appeared to be the cause of the skin fissures. Rapid healing occurred when the applications were discontinued. No evidence of skin sensitization could be demonstrated when albino guinea pigs received PM by intradermal injections daily for 10 days followed by a challenging dose 2 weeks later.

Feeding Studies

When a diet containing 15% PM was fed to rats, cathartic effects were observed. At levels below 5%, the effect was less severe. Rats fed

TABLE 1
EFFECT OF PM, PS, AND GUM GUM ON GROWTH AND FOOD EFFICIENCY

Dietary treatment	Mean weight gain \pm SE after 91 days ^a (g)	Food efficiency (gain/food intake)
Basal diet A	217.4 \pm 6.57	0.16
+ 3% phosphomannan	203.8 \pm 5.51	0.15
+ 6% phosphomannan	188.0 \pm 9.84	0.14
+ 3% polysaccharide B-1450	200.8 \pm 6.66	0.14
+ 6% polysaccharide B-1450	199.2 \pm 1.99	0.14
+ 6% gum guar	204.6 \pm 4.88	0.14

^a Five weanling male rats per group. Mean starting weight = 58.0 g.

large fecal pellets, which were well formed in contrast to the more fluid and sticky consistency of the feces from rats ingesting PM.

Reduced food intakes and decreased growth rates, as well as abnormal feces, were observed when 7.5-15% dietary levels of PM or PS were fed. At lower levels—diets containing 3% or 6% PM, PS, or gum guar—

TABLE 2
EFFECT OF DEXTRAN, AGAR, AND GUM ARABIC ON GROWTH AND FOOD EFFICIENCY

Dietary treatment	Mean weight gain \pm SE after 62 days ^a (g)	Food efficiency (gain/food intake)
Basal diet B	190 \pm 10.4	0.28 \pm 0.006
+ 15% agar	190 \pm 11.9	0.23 \pm 0.006
+ 15% gum arabic	224 \pm 7.8	0.28 \pm 0.003
+ 15% dextran B-512F	197 \pm 13.9	0.27 \pm 0.005

^a Six weanling male rats per group in individual cages. Mean starting weight = 37.5 g.

weight gains were not significantly below those of the unsupplemented group (Table 1).² No significant alterations in hemoglobin or red and white cell counts were observed in these rats. Likewise, no abnormalities

² The statistical test used was Student's *t* test.

TABLE 3
CALORIC AVAILABILITY EVALUATION OF VARIOUS CARBOHYDRATES

Diet	Amount of supplement (g rat day)	Weight change ^a (g)		Fecal weight increase (g dry wt.)	Digestibility ^b (%)
		7 Days on supplement	2 Days on Basal		
Basal (5 g)	0.25	-3	-6	-9	100
+ glucose	0.5	+4	-6	+7	100
+ glucose	1.0	+11	-7	+13	100
+ dextran B-512F	0.4	+21	-12	+18	100
+ phosphomannan Y-2448	0.4	+18	-8	+19	100
+ polysaccharide B-1450	0.4	+19	-16	+12	78
+ gum guar	0.4	+3	-20	-8	0
Basal (5 g)	0.4	+6	-14	+1	76
+ d-glucose	0.5	+15	+3	+18	100
+ d-glucose	1.0	+38	+1	+21	100
+ pectin N.E.	1.0	+33	-18	-3	160
+ D-mannose	0.75	+31	-5	+28	19
Basal (5 g)	0.75	0	-2	-2	92
+ D-glucose	0.5	+7	-2	+7	100
+ D-glucose	1.0	+27	-1	+28	100
+ dextran, low mol. wt.	1.0	+28	+1	+31	99
+ dextran, high mol. wt.	1.0	+21	-5	+16	86
+ gum arabic	0.75	+19	-5	+7.4	71
Average for 5 rats each.					21
^a Digestibility = (total intake of test material minus increase in fecal weight)/total intake of test material.					
^b Two days on basal followed 7 days on supplement.					
^c Adjusted for weight changes in rats on basal diet throughout.					

were noted when organ weights were evaluated. The tissues of rats that had ingested diets containing 15% PM or PS for 91 days were subjected to histologic examination by Dr. William E. Ribelin, veterinary pathologist. There was no evidence of pathology.

Weight gains and food efficiencies were determined for rats ingesting high levels (15%) of agar, gum arabic, and dextran (Table 2). While the food efficiency of the group on agar was significantly lower, the weight gain was nearly normal.

A paired-feeding experiment compared the growth of rats ingesting ad libitum a diet containing 7.5% PS with comparable rats restricted to the same intake of the basal diet reduced by 7.5%. At the end of 18 days the weight gains were identical for the restricted and ad libitum groups, thus indicating the absence of any growth-inhibiting factor.

The weight gains of rats receiving polysaccharides while on a restricted caloric intake are shown in Table 3. For comparison, a group receiving no supplement and groups receiving measured amounts of a standard caloric source such as glucose were included in each trial. D-Mannose was included in these studies because it is a major component of both PM and PS.

The weight gains shown in column 3 of Table 3 suggest that all the polysaccharides fed were utilized to varying degrees. However, in earlier experiments the weight gains of supplemented groups were sometimes higher than was theoretically possible. This was believed to be due to the weight of hydrated undigested residues in the gastrointestinal tract. To check this possible response, the weight losses of rats that were returned to the basal diet for 2 days following the 7-day period of supplementation were determined (column 4). After adjusting for weight changes in rats on the basal diet, net losses or no gain were observed for the PS, gum guar, and pectin-supplemented groups (column 5). These materials, apparently, are not utilized. This conclusion is substantiated in the case of PS and pectin by the finding that practically all the PS and pectin fed during the 7-day period could be accounted for in the feces (column 6). Only in the case of gum guar is there a contradiction, in that digestibility was good (76%), yet utilization based on weight gain was very low (71%).

SUMMARY

Catharsis and reduced rates of growth were the only detectable effects produced in rats ingesting diets containing 15% phosphomannan Y-2448 or polysaccharide B-1450 during a 91-day period. A third microbial polysaccharide, dextran B-512F

did not produce the same effects. Digestibility and caloric availability assays indicate that dextran was highly digestible and utilized as a source of energy for growth, whereas polysaccharide B-1459 was nondigestible, being completely accounted for in the feces. Phosphomannan Y-2448 was partially utilized.

Data on gum guar, pectin, gum arabic, and agar are included for comparative purposes.

Acute toxicity tests of phosphomannan and polysaccharide B-1459 and skin irritation and sensitization tests were negative.

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ACID HYDROLYSIS OF THE POLYSACCHARIDE GUM FROM *Acacia podalyriaefolia* A. CUNN.: MOLECULAR-WEIGHT DISTRIBUTION STUDIES

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ABSTRACT

The course of acid hydrolysis of the polysaccharide gum of *Acacia podalyriaefolia* has been followed by gel chromatography. Elution patterns of samples removed at intervals demonstrate the rapid hydrolysis of arabinofuranoside linkages and subsequent, preferential removal of galactopyranose end-groups. The persistence of certain peaks in the hydrolysate elution-patterns may be explained on the basis of a galactan framework which could equally well be essentially linear or dendritically branched. The hydrolysis rate-constant decreases continuously with increasing degree of depolymerization.

INTRODUCTION

The occurrence of several peaks in the elution pattern obtained on gel chromatography of an hydrolysate (5M sulphuric acid, 96°, 50 h) of the polysaccharide gum of *Acacia podalyriaefolia* has been noted previously^{1,2}. The study presented here was undertaken in an attempt to account for this, to investigate the behaviour on acid hydrolysis of this and other arabinogalactans, and to seek further information on the structure of *A. podalyriaefolia* gum²⁻⁴. The molecular-weight distributions of degradation products of this polysaccharide are not complicated by polymolecularity of the parent polymer^{1,2}.

EXPERIMENTAL

The *A. podalyriaefolia* gum was a sample of the ethanol-precipitated material described earlier^{3,4}.

General methods. — Paper chromatography was carried out by the descending method on Whatman No. 1 paper with the solvent systems (v/v) (a) ethyl acetate–pyridine–water (10:4:3), (b) butyl alcohol–ethanol–water (1:1:1), and (c) butyl alcohol–ethanol–water (4:1:5, upper layer). Sugars were detected with *p*-anisidine hydrochloride in butyl alcohol⁵.

Optical rotations were measured on a Bellingham and Stanley Model A polarimeter.

Reducing power was determined by the Nelson modification⁶ of the Somogyi method⁷, absorbances being measured at 546 nm on a Beckman DB spectrophotometer.

Gel chromatography. — Most of the hydrolysate samples were chromatographed on a column (90 × 1.5 cm) of the polyacrylamide gel Bio-Gel P-300 (Bio-Rad Laboratories). A column (55 × 1.2 cm) of the less porous P-10 gel was used for low molecular weight hydrolysates. Samples were applied in a volume of 1 ml in all cases. Where Bio-Gel P-300 was used, sample concentration varied from 2 to 8 mg/ml but, owing to the concentration-dependence⁸ of polysaccharide elution volumes on Bio-Gel P-10, the concentration of samples chromatographed on this gel was kept within the range 2–3 mg/ml, *i.e.*, that employed in the calibration of the column². Elution was carried out with M sodium chloride⁹. Fractions (1 ml) were assayed for carbohydrate by the phenol-sulphuric acid method¹⁰, absorbances at 490 nm being measured on a Unicam SP600 spectrophotometer. Molecular weights corresponding to peaks in the elution curves were found from calibration plots as described previously^{1,2}.

Partial hydrolysis with 5 mM H₂SO₄. — *A. podalyriaefolia* gum (dry weight 1.88 g) was heated at 96° in 5 mM sulphuric acid (100 ml) for 96 h (pH rose from 2.1 to 2.4), samples (5 ml) being removed at intervals. Each hydrolysate sample was immediately cooled and its optical rotation measured. After neutralization (barium carbonate) and centrifugation, the samples were examined by chromatography on paper and on Bio-Gel P-300. The reducing power of each hydrolysate was determined in duplicate, 1-ml aliquots being diluted so that the concentration of reducing sugar lay within the range 25–250 µg/ml. The degree of scission (α) of the polysaccharide in each case was calculated by dividing the reducing power (expressed as g of reducing sugar/g of polysaccharide) in excess of that of the gum itself by that of a sample of the gum subjected to prolonged hydrolysis (0.5M sulphuric acid, 96°, 18 h).

Further hydrolysis with 50 mM H₂SO₄. — The acid concentration in the solution (15 ml) remaining after the treatment with 5 mM sulphuric acid was adjusted to 50 mM by the addition of 0.5M sulphuric acid (*ca.* 2 ml), and the solution was heated for a further 20 h at 96° (pH 1.1 to 1.3). Samples (3 ml) removed at intervals were examined as described above, except that the hydrolysates obtained after 8 h or more were chromatographed on Bio-Gel P-10.

RESULTS AND DISCUSSION

Neutral sugar components of hydrolysates. — The only sugar detected on paper chromatography (solvents *a*, *b*, and *c*) of the hydrolysates obtained after treatment of the gum with 5 mM sulphuric acid for 8 h or less was arabinose. Galactose and a trace of rhamnose were found after 12 h, but oligosaccharides only after 24 h. However, the response to the spray reagent of a series of sugars [β -galactose, 6-*O*- β -D-galactopyranosyl-D-galactose, and the corresponding β -D-(1→6)-linked trisaccharide], present in a mixture in equal amounts, was found to decrease markedly with increasing degree of polymerisation. It is possible, therefore, that oligosaccharides, in amounts not detectable by paper chromatography, are produced at an earlier stage of hydrolysis.

The use of solvent *a* permitted the resolution of several oligosaccharide components in the hydrolysates obtained on treatment of the gum with 5 mM sulphuric acid for 24 to 96 h. Two disaccharides, identified as 3- and 6-*O*- β -D-galactopyranosyl-p-galactose on the basis of their chromatographic mobility (R_F 0.42 and 0.35, respectively), were detected, and five higher oligosaccharides (R_F 0.28, 0.17, 0.12, 0.08, and 0.04) were also resolved. The linearity of a plot of the values of $\log [(1/R_F) - 1]$ for these components and for D-galactose and the β -(1 \rightarrow 3)-linked D-galactose disaccharide against the suspected degree of polymerisation (hexose units) is consistent¹¹ with the behaviour of a homologous series of β -(1 \rightarrow 3)-linked D-galactose oligosaccharides.

This series of oligosaccharides was also detected (paper chromatography, solvent *a*) in the hydrolysates obtained on further treatment of the degraded gum with 50 mM sulphuric acid for 8 h or less. After 20 h, sugars higher than the trisaccharide were no longer present in detectable amounts.

Molecular-weight distributions. — The elution patterns obtained on gel chromatography of the hydrolysate samples are shown in Figs. 1–3. The values of \bar{M}_w , the weight-average molecular weight¹², calculated in each case from the molecular weights corresponding to peaks in the elution curve and the relative areas beneath these peaks, are given in Tables I and II, together with the specific rotations and values of the degree of scission, x , for the various hydrolysates.

The elution patterns of samples withdrawn during the initial stages of hydrolysis (Fig. 1, *A–C*) reflect the usual, rapid hydrolysis of the acid-labile arabinofuranoside linkages^{13,14} in the gum. Since these linkages are almost exclusively peripheral in the *A. podalyriaefolia* gum polysaccharide⁴, the initial decrease in \bar{M}_w with increasing x is small. Calculations based on the known³ arabinose content of the gum (mole % 16) predict a molecular weight of 27,200 after hydrolysis of all arabinofuranoside linkages; this agrees with the values (27,500 and 26,500) estimated from the elution volumes of the second polysaccharide peak in *B* and *C* (Fig. 1), respectively.

A striking change in the gel-chromatography elution-pattern occurs after treatment of the gum with 5 mM sulphuric acid for 12 h (Fig. 1, *D*). The peak corresponding to a molecular weight of 31,000 (largely undegraded gum) disappears, that at molecular weight 26,500 is considerably attenuated, and a large peak appears at molecular weight 17,800.

The molecular weight of the product obtained after removal of arabinose and all galactopyranose units present as end-groups or in short branches (3 units or less) in the *A. podalyriaefolia* gum polysaccharide may be estimated on the basis of the degree of branching of the molecule, determined by methylation analysis³. Calculations based on a dendritically branched model of the galactan framework give a value of 17,600, while the use of a model consisting of an essentially linear chain with multiple short-branches predicts a similar value, agreeing well with the molecular weight (17,800) corresponding to the large peak in Fig. 1, *D*. The occurrence of this peak is therefore consistent with the preferential hydrolysis of terminal galactopyranose residues, and those in short branches which rapidly become

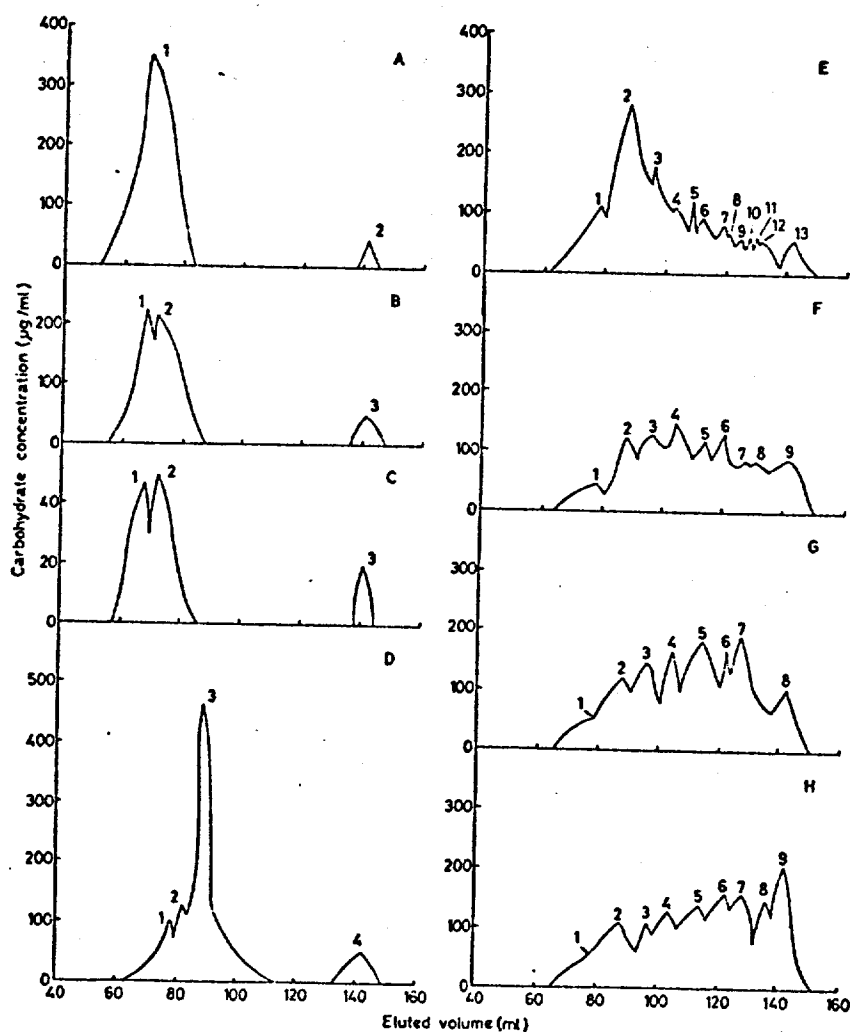


Fig. 1. Bio-Gel P-300 elution patterns (A-H) of hydrolysates of gum treated with 5 mM sulphuric acid at 96°C. For each, there follow duration of hydrolysis and molecular weights corresponding to peaks as numbered: A. 2 h: 1, 31,000; 2, $\leq 1,800$. B. 5 h: 1, 31,000; 2, 27,500; 3, $\leq 1,800$. C. 8 h: 1, 31,000; 2, 26,500; 3, $\leq 1,800$. D. 12 h: 1, 26,500; 2, 22,400; 3, 17,800; 4, $\leq 1,800$. E. 24 h: 1, 26,500; 2, 17,800; 3, 13,000; 4, 10,000; 5, 7,800; 6, 6,700; 7, 5,300; 8, 4,900; 9, 4,200; 10, 3,700; 11, 3,400; 12, 3,200; 13, $\leq 1,800$. F. 48 h: 1, 26,500; 2, 17,800; 3, 13,000; 4, 9,400; 5, 6,500; 6, 5,000; 7, 3,800; 8, 3,300; 9, $\leq 1,800$. G. 72 h: 1, 26,500; 2, 17,800; 3, 13,000; 4, 9,400; 5, 6,500; 6, 4,500; 7, 3,700; 8, 2,700; 9, $\leq 1,800$. H. 96 h: 1, 26,500; 2, 17,800; 3, 13,000; 4, 9,400; 5, 6,500; 6, 4,500; 7, 3,700; 8, 2,700; 9, $\leq 1,800$.

terminal after such hydrolysis. The now considerable body of evidence in favour of the view that acid hydrolysis of terminal linkages in polysaccharides is more rapid than that of internal bonds has recently been reviewed by BeMiller¹⁵.

The elution pattern obtained after treatment of the gum with 5 mM sulphuric acid for 24 h (Fig. 1, E) shows, in addition to the peaks at molecular weights 26,500 and 17,800, which have decreased in magnitude, several small peaks at elution volumes

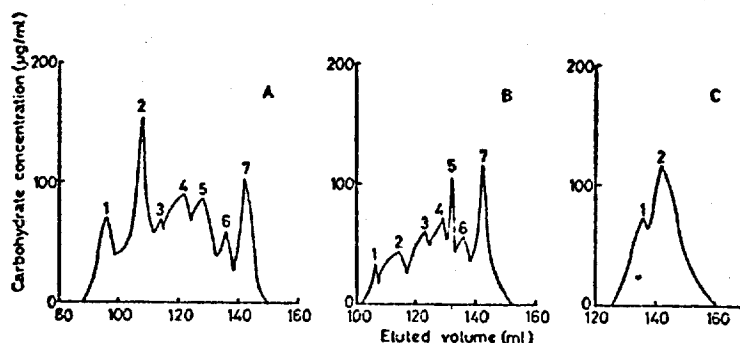


Fig. 2. Bio-Gel P-300 elution patterns (A-C) of hydrolysates obtained on treatment of partially hydrolysed gum with 50 mM sulphuric acid at 96°. A. 1 h: 1, 13,000; 2, 8,400; 3, 6,500; 4, 4,700; 5, 3,700; 6, 2,700; 7, $\leq 1,800$. B. 3 h: 1, 8,800; 2, 6,500; 3, 4,500; 4, 3,500; 5, 3,200; 6, 2,700; 7, $\leq 1,500$. C. 8 h: 1, 2,700; 2, $\leq 1,800$.

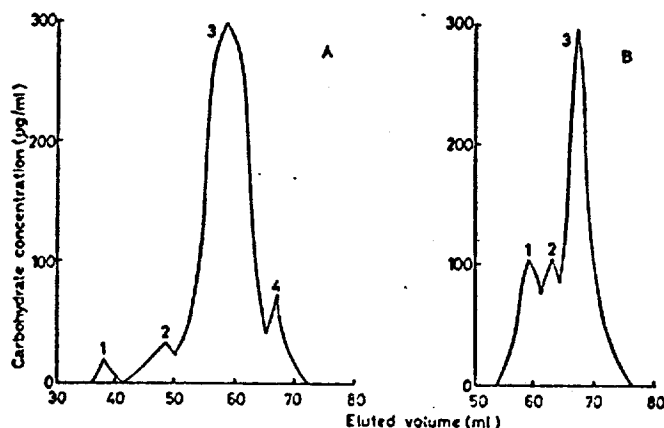


Fig. 3. Bio-Gel P-10 elution patterns (A, B) of hydrolysates obtained on treatment of partially hydrolysed gum with 50 mM sulphuric acid at 96°. A. 8 h: 1, 2,700; 2, 1,300; 3, 500; 4, ≤ 250 . B. 20 h: 1, 490; 2, 350; 3, ≤ 250 .

TABLE I

PARTIAL HYDROLYSIS OF *A. podalyriaefolia* GUM BY 5mM SULPHURIC ACID

Time of hydrolysis (h)	Degree of scission, α	$[\alpha]_D^{20}$ (degrees) ^a	\bar{M}_w	$10^5 k$ (sec ⁻¹)
0	—	+5.5	31,500 (Ref. 1)	
2	0.009	+6.9	31,000	1.25
5	0.022	+12	29,000	1.24
8	0.033	+16	28,200	1.04
12	0.046	+21	19,800	0.944
24	0.077	+31	14,800	0.762
48	0.112	+37	10,600	0.421
72	0.136	+40	10,000	0.344
96	0.156	+41	9,100	0.272

^aIn 5mM sulphuric acid (c 1.88).

TABLE II
FURTHER HYDROLYSIS OF GUM BY 50MM SULPHURIC ACID

Time of further hydrolysis (h)	Degree of scission, x	$[\alpha]_D^{20}$ (degrees) ^a	\bar{M}_w	$10^6 k$ (sec ⁻¹)
1	0.359	+48	5,700	
3	0.500	+54	3,500	34.5
8	0.660	+61	550	26.9
20	0.786	+72	280	8.44

^aIn 50mm sulphuric acid (c 1.66).

corresponding to lower molecular weights. As the time of hydrolysis increases from 24 to 96 h, peaks at molecular weights of *ca.* 26,500, 17,800, 13,000, 9,400, 6,500, 4,700, and 3,800, as well as the sugar peak ($\leq 1,800$), persist in the elution patterns (Fig. 1, *F-H*), the low molecular-weight peaks growing at the expense of those corresponding to higher molecular weights with increasing degree of hydrolysis. On further treatment of the degraded gum with 50mm sulphuric acid, the polysaccharide peaks gradually disappear (Fig. 2); chromatography on Bio-Gel P-10 shows the hydrolysates obtained after 8 h or more to consist of mixtures of sugars (Fig. 3).

Apart from the values of 26,500 and 17,800, which have been discussed, the molecular weights corresponding to the persistent peaks in the hydrolysate elution-patterns are all approximately half of the values associated with peaks at lower elution volumes (*e.g.* the series 26,500, 13,000, 6,500). It is possible, therefore, that the occurrence of several peaks, rather than a single, broad peak at a molecular weight corresponding to \bar{M}_w for the appropriate degraded polysaccharide, is simply a reflection of the fact that, on scission of a middle linkage in a chain, two fragments of equal size are produced by breaking only one bond. High yields of such fragments may thus be expected at the appropriate degree of scission^{16,17}.

Various statistical treatments of the depolymerization of long-chain molecules have been published¹⁶⁻¹⁹. Montroll and Simha¹⁷ considered the decrease of \bar{M}_w with increasing degree of scission, x , in the case of completely random depolymerization and derived the equation

$$\frac{\bar{M}_w}{M_0} = \frac{nx^2 + 2(1-x)[(1-x)^n + nx - 1]}{nx^2} \quad (1)$$

where M_0 = molecular weight of monomer unit, and n = number of monomer units in undegraded polymer.

Simha¹⁸ subsequently considered the case of preferential scission of terminal linkages, the rate constant for the breaking of internal linkages being regarded as negligible in comparison with that for terminal bonds; thermal depolymerization of polystyrene in the liquid phase at 350° was cited as an example of a reaction believed

proceed in this way. For this situation, he derived the equation

$$\frac{\bar{M}_w}{M_o} = 1 + e^{-z} \left[(n-1) \frac{z^{n-2}}{(n-2)!} + \left(n-2z-1 + \frac{2z}{n} \right) \frac{z^{n-3}}{(n-3)!} \right. \\ \left. + \left(1 - \frac{z}{n} \right) \left(n-z-1 + \frac{z}{n-z} \right) \left(e^z - \sum_{r=n-3}^{\infty} \frac{z^r}{r!} \right) \right] + \frac{2R}{n}, \quad (2)$$

where $z = 2kt$, k being the hydrolysis rate-constant at time t ,

$$R = e^{-z} 2^{n-2} \sum_{i=n-1}^{\infty} \left(\frac{z}{2} \right)^i \frac{1}{i!},$$

and M_o and n have the same significance as in equation (1).

The variation of \bar{M}_w/M_o with x on progressive acid-degradation of the *A. podalyriaefolia* gum polysaccharide, as determined in the present work*, is shown in Fig. 4 (curve 1). Fig. 4 (curve 2), which would be obtained if hydrolysis were almost exclusively confined to terminal linkages in the initial stages ($x \leq 0.156$), has been calculated from equation (2), the value of z given by the values of k (see below) and t at the appropriate x (Table I) being inserted into the equation in each case. Fig. 4 (curve 3), calculated from equation (1), shows the \bar{M}_w/M_o values predicted if depolymerization were completely random after hydrolysis of the arabinofuranoside linkages was essentially complete. In this case, the molecular weight of the parent polymer was taken as 27,200 (the theoretical value for the product obtained after removal of all arabinose from the gum), and the x values inserted into equation (1) were calculated with the value (0.038, from Fig. 4, curve 1) corresponding to this \bar{M}_w as the origin.

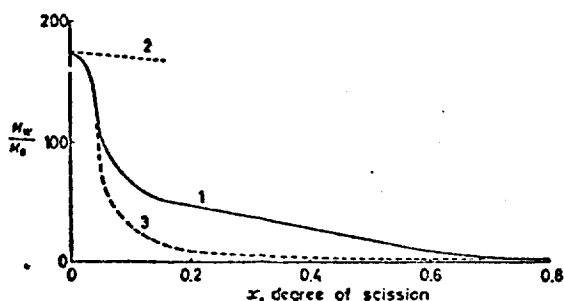


Fig. 4. Variation of \bar{M}_w/M_o with degree of scission. 1. Experimental values. 2. Values predicted by equation (2). 3. Values predicted by equation (1).

It is evident from Fig. 4 that the experimentally determined values of \bar{M}_w/M_o deviate from curve 2 to an increasing extent as x increases and hydrolysis of peripheral arabinofuranoside linkages, approaching completion, has less dominance in the

* \bar{M}_w/M_o has been calculated on the basis of a value of 180 for M_o . Allowance for varying proportions of arabinose in the polysaccharide has a negligible effect upon the calculated values of \bar{M}_w/M_o , owing to the low arabinose content of the gum.

overall hydrolysis. However, the experimental curve lies above curve 3 over most of its length; the two curves coincide only at high x (≥ 0.74). Hydrolysis of this polysaccharide is clearly non-random, even after cleavage of most of the arabinofuranoside linkages. Reasons for this include the faster hydrolysis of terminal than internal galactopyranose residues, as suggested above, and the presence of both β -(1 \rightarrow 3) and β -(1 \rightarrow 6) linkages between D-galactopyranose residues^{2,3}. The hydrolysis rates of these linkages in the D-galactobioses are appreciably different²⁰ [β -(1 \rightarrow 3) faster than β -(1 \rightarrow 6)], and differences might therefore be expected in the case of the polysaccharide also. Furthermore, both chain units and branch-points are present in comparable amounts².

Kinetics of hydrolysis. — The mean values of the hydrolysis rate-constant, k , over the successive time-intervals between samples are given in the extreme right-hand columns of Tables I and II. These values were calculated from the equation

$$k = \frac{1}{t_2 - t_1} \ln \frac{1 - x_1}{1 - x_2},$$

where t_1 and t_2 are the times of hydrolysis corresponding to consecutive measured values, x_1 and x_2 , of the degree of scission.

It is clear that, under given conditions, k decreases with increasing x . This is in contrast to the acid hydrolysis of cellulose²¹ and amylose²², in which an increase in the rate constant, attributed to increasing proportion of end-groups, is observed as hydrolysis progresses. In the present work, the effect of any increase in the proportion of end-groups will be small in comparison with the decrease in hydrolysis rate which must occur as the rapid hydrolysis of arabinofuranoside linkages approaches completion and the overall rate of hydrolysis becomes increasingly dependent upon the slower disintegration of the galactan framework of the polysaccharide.

The relative magnitudes of these two effects may be assessed by comparing rate constants given in the literature. Myrbäck and Magnusson²³ have calculated that the end-groups in starch are hydrolysed 1.68 times more rapidly than the internal linkages; in the case of cellulose, a ratio of *ca.* 3:1 has been estimated²¹. In contrast, comparison of values reported for the hydrolysis constants of various methyl glycosides^{15,24} shows that methyl β -D-galactopyranoside is hydrolysed 17 times more slowly than methyl α -L-arabinofuranoside under the same conditions. (The initial increase in specific rotation on acid hydrolysis of *A. podalyriaefolia* gum is consistent with the presence of α -L-arabinofuranoside linkages.) The value found for k in the early stages of the treatment of the gum with 5M sulphuric acid ($1.25 \times 10^{-6} \text{ sec}^{-1}$, from Table I) is, however, much lower than that reported²⁴ for the hydrolysis of methyl α -L-arabinofuranoside in 5M acid, at 100° ($260 \times 10^{-6} \text{ sec}^{-1}$)*. Furthermore, the decrease in k during 96 h is less than expected from the values of k for the respective methyl glycosides²⁵. Hydrolysis rates of linkages in polysaccharides are not strictly comparable with those of the same linkages in simple glycosides, owing to

*The value given in the reference cited is based on common logarithms; here it has been converted into that based on natural logarithms, in accordance with current practice¹⁵.

steric and other factors¹⁵. The rate of hydrolysis of the arabinofuranoside linkages, however, remains higher than that of galactopyranoside. Moreover, in the case of a molecule as highly branched as the *A. podalyriaefolia* gum polysaccharide³, the proportion of end-groups will not necessarily increase with decreasing degree of polymerization; with the removal of short side-chains as hydrolysis progresses, it is more likely to decrease. The continuous decrease in hydrolysis rate-constant with increasing degree of scission of the polysaccharide, when the hydrolysis conditions remain constant, is therefore not unexpected.

The values found for k on hydrolysis of the partially degraded polysaccharide by 50mM acid (Table II) are all considerably lower than those for hydrolysis of the α -galactobioses under comparable conditions [$ca. 200 \times 10^{-6}$ and $80 \times 10^{-6} \text{ sec}^{-1}$ for the β -(1 \rightarrow 3) and β -(1 \rightarrow 6) linked isomers, respectively, from the half-hydrolysis times²⁰]. This may be attributed to the retarded hydrolysis of inner bonds in the galactan framework; cleavage of the peripheral linkages possibly proceeds at a rate comparable with that of hydrolysis of the bioses¹⁵, except at branch-points²⁶.

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Identification of Stabilizing Agents

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EXTENSIVE use of a variety of polysaccharides as stabilizing or thickening agents in foods has created a need for analytical methods for the identification of commercially available materials and for their detection and quantitative estimation in food products. A number of stabilizers, which are used or have been suggested for use in foods, are listed and their chemical nature is indicated in Table I. Throughout this report the term "gums" is used in referring to all of these materials. Gelatin is included because it has many uses similar to those of the polysaccharides.

As the initial step in a project concerned with the development

of the required analytical procedures, methods for the qualitative identification of a number of gums have been studied. Several procedures for the identification of gums are found in the literature. A method described by Jacobs and Jaffe (15) classifies the gums on the basis of physical characteristics or appearance of precipitates and thus requires considerable experience on the part of the analyst. Their outline does not include pectic substances, alginates, methylcellulose, carboxymethylcellulose, gum ghatti, starch, or gelatin. It also has the disadvantage of using an unstable reagent (Millon's) which must be freshly prepared each day. A method developed by Cannon and

The present work was undertaken to develop a method for the identification of stabilizing and thickening agents used in food products. The materials studied were pectin, de-esterified pectin, algin, Irish moss, gum tragacanth, gum karaya, locust bean gum, starch, agar, gum arabic, gum ghatti, carboxymethylcellulose, methylcellulose, and gelatin. A proposed identification scheme is based on precipitation reactions with calcium chloride,

sodium hydroxide, barium hydroxide, and lead acetate. In addition, reactions of the stabilizing agents with a cationic soap, ammonium sulfate, mercuric nitrate, papain, and gelatin are listed. The proposed scheme should be useful for identification of unknown stabilizing agents. A number of the reactions reported might be employed for the identification of individual stabilizing agents in mixtures of these materials or isolated from foods.

the Association of Official Agricultural Chemists (3, 5) does not provide for the identification of pectic substances, alginates, methylcellulose, carboxymethylcellulose, locust bean (carob) gum, or gum ghatti. Bryant (4) has described a procedure for distinguishing between pectin and certain gums, but it does not provide for positive identification of the gums. A number of other publications dealing with characteristic properties of these polysaccharides have been summarized by Mantell (16), but a systematic procedure is still needed for their identification. Such a procedure would be useful for the identification of products used as thickeners or stabilizers in foods, drugs, and cosmetics and ultimately for the identification of polysaccharides isolated from these materials.

In the present investigation, the manner in which the gums disperse in water after being wetted with alcohol has been a valuable index to the identity of unknown samples. Their solubility properties are summarized in Table II. Use has also been made of the fact that many of the polysaccharides occur as salts of complex organic acids (Table I). The acidic properties may be due to the presence of uronic acid groups, as in gum arabic, or to the unesterified portion of sulfuric acid molecules esterified with the polysaccharide. When mineral acids are added to aqueous solutions or dispersions of these salts, the effective concentrations of the polysaccharide anions are decreased. Thus, although the complex anions may yield insoluble salts with heavy

metal cations, most are not precipitated from acid solutions. The amount the pH must be raised in order to precipitate the heavy metal salts—e.g., barium, mercury, or lead—is frequently characteristic of the individual polysaccharide.

EXPERIMENTAL

During the present investigation 0.5 to 1.0% aqueous dispersions of the polysaccharides were used for the tests. Aliquots of from 3 to 5 ml. were treated with varying concentrations of the reagents which it was hoped would give characteristic precipitation reactions. Initially the reagents used were those for which Jacobs and Jaffe (15) have described reactions with several polysaccharides. Subsequently a number of other reagents were used.

Table II. Dispersion in Water of Gums, Wetted with Alcohol

Gum	Manner of Dispersal in Water
Pectic acid	Insoluble
Pectate (Na, K, or NH ₄ salts)	Forms either clear or turbid solution on heating
Pectate (Ca salts)	Insoluble
Pectin	Swells in cold water and dissolves on heating
Alginate	Dissolves slowly in cold water or quickly on heating to form viscous solution
Irish moss	Dissolves slowly in cold water, rapidly on heating to form viscous solution
Agar	Swells in cold water, dissolves on heating, gels on cooling
Tragacanth	Swells to form viscous dispersion in cold or hot water, but does not form true solution
Methylcellulose	Dissolves slowly in cold water but becomes cloudy or gels on heating
Starch	Disperses on heating
Carboxymethylcellulose	Dissolves slowly in cold water, rapidly on heating, giving clear viscous solution with some fine fibrous suspended material
Locust (carob)	Forms viscous suspension but not a true solution
Karaya	Forms viscous suspension. Insoluble particles settle on standing
Arabic (acacia)	Dissolves in cold water to form a clear only slightly viscous solution
Ghatti	Dissolves to form almost clear solution but some insoluble material may remain as fine suspension
Gelatin	Swells in cold water and dissolves on heating

Table I. Source and Chemical Nature of Materials Commonly Used as Thickening Agents in Foods

Material	Source	Principal Components	References
Pectic substances	Fruits	Galacturonic acid (occurs as methyl ester)	(18)
Algin (sodium alginate)	Seaweeds	Mannuronic acid (Na salt)	(16)
Irish moss	Seaweeds	Galactose, galactose 4-sulfate (K and Ca salts)	(18)
Agar	Seaweeds	Galactose (D- and L-), galactose 6-sulfate (Ca and Mg salts)	(18)
Tragacanth	Plant gum	L-Fucose, D-xylose, galacturonic acid, L-arabinose, D-galactose	(18)
Methylcellulose	Modified cellulose	Methyl-D-glucose	(18)
Starch	Plants	D-Glucose	(18)
Carboxymethylcellulose	Modified cellulose	Carboxymethyl-D-glucose	(18)
Locust bean gum (carob gum)	Seed endosperm	Mannose and galactose	(16)
Guar gum	Seed endosperm	Mannose and galactose	(20)
Karaya	Plant gum	Galactose, acetic acid, galacturonic acid, rhamnose, tagatose	(14)
Arabic (acacia)	Plant gum	D-Glucuronic acid, D-galactose, L-arabinose, rhamnose (mixed Ca, Mg, and K salts)	(18)
Ghatti	Plant gum	L-Arabinose, galactose, galacturonic acid (Ca salt)	(16)
Gelatin	Modified protein	Amino acids	

Reactions which were found useful for characterizing the gums are summarized in Tables III and IV. Only those materials having anionic components, such as alginates, or potential anionic components, such as pectin, give pronounced reactions with cationic soap (Table III). As in the case of precipitates with heavy metals, the precipitates with the cationic soap quickly disperse on acidification of the medium. Ammonium sulfate is of interest, in that it gives pronounced precipitation tests with several of the gums but not with alginates, pectin, tragacanth, karaya, arabic, or ghatti, each of which probably contains uronic acid components. The reactions with Stokes's acid mercuric nitrate illustrate the effects of low pH on precipitation of heavy metal salts of the polysaccharide acids. An excess of the reagent makes the solutions strongly acidic and thus the weakly dissociated acids redisperse. Alginic acid and pectic acid are insoluble and thus are not dissolved by excess Stokes reagent. Papain and gelatin give pronounced precipitation reactions only with those gums having anionic components. These precipitates are found only if the

Table III. Precipitation Reactions of Polysaccharide Gums and Gelatin

Gum	1 Vol. 1% Solution of Cationic Soap ^a	0.5 Vol. Saturated Ammonium Sulfate	Diluted ^b Stokes's Acid Mercuric Nitrate Added Dropwise	1 Vol. 2% Papain (6) ^c	1 Vol. 2% Gelatin ^c	4 Vol. 95% C ₂ H ₅ OH + 2 - 3 Drops Saturated NaCl
De-esterified pectin	Fine opaque precipitate	Gelatinous translucent precipitate	Gels (almost opaque). Insoluble in excess reagent	Precipitate	Precipitate	Gelatinous precipitate, gels (1 vol.)
Alginate	Fine opaque precipitate	Nil	Gels (almost opaque). Insoluble in excess reagent	Precipitate	Precipitate	Gelatinous precipitate (1 vol.) becomes stringy with 4 vol. alcohol
Pectin	Flocculent precipitate	Nil	Forms almost opaque gel which dissolves in excess reagent	Cloudy	No definite effect	Transparent gelatinous precipitate. Gels (1 vol.)
Irish moss	Stringy or flocculent precipitate	Gelatinous precipitate or gel	Transparent gel. Redispersed by excess reagent	Precipitate	Precipitate	Stringy precipitate
Agar	Gelatinous precipitate	Flocculent precipitate	Turbid or cloudy	Cloudy	Precipitate	Fine flocculent precipitate
Tragacanth	Fine opaque precipitate	Nil	Flocculent precipitate. Dissolves in excess reagent	Precipitate	Precipitate	Voluminous precipitate, jellylike
Methylcellulose	Nil	Precipitate	Nil	Nil	Nil	Nil
Starch	Nil	Precipitate	Nil	Nil	Nil	Opaque flocculent precipitate
Carboxymethylcellulose	Gelatinous clotted precipitate	Gelatinous precipitate	Precipitate dissolves in excess reagent	Precipitate	Precipitate	Voluminous clotted precipitate
Locust	Nil	Precipitate (voluminous)	No pronounced effect	No pronounced effect	Nil	Voluminous opaque stringy precipitate, forms clot
Karaya	Flocculent precipitate	Nil	Flocculent precipitate dissolves in excess reagent	Precipitate	Precipitate	Flocculent precipitate, discrete particles
Arabic (acacia)	Precipitate (very fine)	Nil	Flocculent precipitate dissolves in excess reagent	Precipitate	Precipitate	Fine opaque nonsettling precipitate
Ghatti	Fine precipitate	Nil	Nil	Nil	Precipitate	Fine precipitate, nonsettling (2-3 vol.)
Gelatin	Precipitate in alkaline medium (22)	Precipitate	Nil	Nil	Nil	Finely flocculent precipitate, coagulates

^a Rodalon (alkyl dimethyl benzyl ammonium chloride), Fairfield Laboratories, Plainfield, N. J.

^b Mercury dissolved in twice its weight of concentrated nitric acid and diluted to 100 times its volume with distilled water.

^c Precipitates with papain and gelatin are observed only in weakly acidic medium and most exhibit properties of coacervates rather than true precipitates.

pH of the mixture is below the isoelectric point of the protein and it is possible that they would be more correctly called coacervates. They are usually dispersed by a few drops of mineral acid or of dilute ammonium hydroxide. The characteristic manner in which some of the gums are precipitated by alcohol may also be of value in their identification.

The reactions described in Table IV form the basis of a proposed procedure for the systematic identification of the gums.

REAGENTS

Calcium chloride (CaCl₂), 3% solution (weight/volume).

Ammonium hydroxide, 3.0 *N* solution.

Sodium hydroxide, 3.0 *N* solution.

Barium hydroxide, saturated solution stored in a bottle equipped with a siphon and a soda-lime tube.

Basic lead acetate, 20% suspension (weight/volume). Heat to boiling, cool, and use supernatant solution.

Hydrochloric acid (or other mineral acid), 3.0 *N* solution.

Methylene blue, 0.1% aqueous solution.

Tincture of iodine (U.S.P., 14).

Iodine-potassium iodide stock solution, containing 0.5% iodine and 1.0% potassium iodide. Iodine-potassium iodide test solution, consisting of stock solution diluted 1 to 5.

Cupric sulfate (CuSO₄ · 5H₂O), 15% solution (weight/volume).

Borax (Na₂B₄O₇ · 10H₂O), 4% solution.

Ruthenium red test solution (3).

Picric acid, saturated aqueous solution.

IDENTIFICATION PROCEDURE

Wet a 0.25- to 0.5-gram sample of the material to be identified with 1 to 2 ml. of 95% alcohol and add 50 ml. of distilled water. Suspend the solid material in the water by shaking or stirring. Heat the suspension, with frequent shaking, on a hot plate or over a burner. If the sample dissolves, discontinue heating immediately; otherwise hold at 85° to 95° C. for 15 minutes.

Group A. I. Treat a 3- to 5-ml. aliquot of the solution with 0.2 volume of 0.25 *M* calcium chloride. A gelatinous precipitate or gel indicates alginates or de-esterified pectin.

If no reaction is apparent with calcium chloride alone, add 1 vol-

ume of 3 *N* ammonium hydroxide to the calcium chloride treated sample. Slow formation of a gel or gelatinous precipitate indicates pectin.

II. If either test in A I was positive, mix a fresh 3- to 5-ml. aliquot of sample with 1 volume of 3.0 *N* sodium hydroxide. Observe the reaction and then heat the mixture in a boiling water bath for 10 minutes.

Immediate formation, in the cold, of a gelatinous or flocculent precipitate indicates either pectin or de-esterified pectin. No precipitate indicates alginates. All three mixtures become yellow on heating, but the precipitates with pectic substances do not dissolve.

Group B. If the material is not an alginate or a pectic substance, carry out the following tests.

I. Mix a 3- to 5- ml. aliquot of the sample with 0.1 volume of saturated barium hydroxide. Observe in the cold and heat in boiling water bath for 10 minutes.

Formation in the cold of a nonsettling, almost opaque, gelatinous precipitate indicates Irish moss. Carry out confirmatory test for Irish moss.

A small amount of flocculent precipitate or cloudiness in the cold and a definite lemon yellow color on heating identify gum tragacanth.

Color changing during heating to yellow, then to green, and finally to gray indicates agar. Carry out confirmatory test for agar.

If the mixture becomes cloudy or forms a gel on heating, but becomes clear on cooling, methylcellulose is indicated. Carry out confirmatory test for methylcellulose.

An opaque flocculent precipitate which may tend to redispense on heating and reprecipitate on cooling indicates starch. Carry out confirmatory test for starch.

Precipitates which disappear when the barium hydroxide is thoroughly mixed with the sample may be disregarded at this point.

II. If the material has not been identified, mix a fresh 3- to 5-ml. aliquot of sample with 1 volume of saturated barium hydroxide. Observe whether there is an immediate precipitation and examine again after standing 5 minutes at room temperature.

Table IV. Precipitation Reactions Used as Basis of Proposed Procedure for Identification of Gums

(Reactions used as identification tests within heavy lines)

Gum	1/2 Vol. 2.5% $\text{CaCl}_2 + 1/10$ Vol. 3 N $\text{Ni}(\text{OH})_2$	1/10 Vol. Saturated $\text{Ba}(\text{OH})_2$ Heated	1 Vol. Saturated $\text{Ba}(\text{OH})_2$	1/2 Vol. Basic Lead Acetate	Basic Lead Acetate + 1/10 Vol. 3 N $\text{Ni}(\text{OH})_2$	Confirmatory Tests
Deacetylated pectin	Gelatinous ppt. or gel	Gel or gelatinous ppt.	Ppt. and soln. turn yellow	Gels	As for lead acetate alone	Gelatinous ppt. when acidified with mineral acids
Alginate	Gelatinous ppt. or gel	Gel or gelatinous ppt.	Ppt. and soln. turn yellow	Gels	As for lead acetate alone	Gelatinous ppt. when acidified with mineral acids
Pectin	Gelatinous ppt. or gel	Gel or gelatinous ppt.	Ppt. and soln. turn yellow	Translucent gel	As for lead acetate alone	
Irish moss	Some samples may give flocculent ppt.	Gelatinous ppt. or gel, almost opaque	Ppt. may flocculate with prolonged heating	Gelatinous ppt. or gel	As for lead acetate alone	Forms blue fibrous ppt. with aqueous methylene blue
Tragacanth		May be small amount of ppt.	Leaves yellow color	Flocculent ppt.	As for lead acetate alone	
Agar		Nil	Becomes yellow then green and gray	Flocculent ppt.	Gels	Gives blue or black stain with tincture of iodine
Methylcellulose		Nil	Becomes turbid or forms gel; becomes clear on cooling	Nil	Gels	Aqueous dispersions are not precipitated by alcohol, but form gel or become cloudy when heated
Starch		Opaque flocculent ppt.	May redisperse	Flocculent ppt.	Very heavy flocculent or gelatinous ppt.	Blue stain with I-KI reagent
Carboxymethyl-cellulose		Ppt. dissolves on shaking		Gels	As for lead acetate alone	Forms clotted ppt. with CuSO_4
Locust*				Opaque gel	As for lead acetate alone	Gels with 1/10 vol. 4% borax
Karaya				Flocculent ppt.	As for lead acetate alone	Swells and stains pink with ruthenium red test solution
Arabic (acacia)				Voluminous opaque ppt.	As for lead acetate alone	Readily soluble in water at room temperature
Ghatti				May be small amount of flocculent ppt.		Fine ppt. with 4 vol. of alcohol. Arabic also gives fine ppt.
Gelatin						Gives fine yellow ppt. when added to saturated picric acid

* Locust bean gum and guar gum give identical reactions and cannot be distinguished on basis of these tests.

Table V. Samples Examined by Proposed Identification Procedure

Gum	Type	No. of Samples
Sodium pectate	Powdered	1
Pectic acid	Granular	1
Pectin	Powdered	2
Sodium alginate	Powdered	5
Irish moss	Powdered	2
Tragacanth	Powdered	1
Agar	Shredded	1
	Granular	3
Methylcellulose	Fibrous	2
Starch	Whole wheat flour	1
	Tapioca flour	1
	Soluble starch	3
	Cornstarch	1
	Amioca (amylopectin) ^a	1
	Clear jel ^a	1
	Clear-Flo-II (sodium salt of starch acid ester) ^a	1
	Dry-Flo (starch ester) ^a	1
	Vulca (starch ether) ^a	1
	Melojel ^a	1
	Nu-film (starch acid ester) ^a	1
Sodium carboxymethyl-cellulose	Powdered	1
Locust bean (carob)	Powdered	7
Guar	Powdered	1
Karaya	Powdered	1
Arabic (acacia)	Powdered	3
	Lump	1
Ghatti	Powdered	1
	Lump	1
Gelatin	Granular	2

^a Products of National Starch Products, New York, N. Y.

A voluminous opaque, stringy precipitate which tends to form a clot indicates locust bean gum. This precipitate may appear flocculent if the mixture is shaken vigorously. Carry out confirmatory test for locust bean gum.

A voluminous opaque flocculent precipitate which forms immediately indicates carboxymethylcellulose. Carry out confirmatory test for carboxymethylcellulose.

An opaque flocculent precipitate which forms slowly and is not voluminous indicates gum karaya. Carry out confirmatory test for karaya.

Group C. If the sample has not been identified it may be gum arabic, gum ghatti, or gelatin.

I. Mix a fresh 3- to 5-ml. aliquot of sample with 1 ml. of basic lead acetate solution. Immediate formation of a voluminous opaque precipitate indicates gum arabic.

If there was only a small amount of flocculent precipitate, or no precipitate, with the basic lead acetate add 1 ml. of 3.0 *N* ammonium hydroxide to the lead-containing sample. A voluminous opaque flocculent precipitate indicates gum ghatti. If there is no precipitate, the sample probably is gelatin. Carry out confirmatory test for gelatin.

CONFIRMATORY TESTS

ALGINATES AND DE-ESTERIFIED PECTINS. Add 0.2 volume of 3 *N* hydrochloric acid (or other mineral acid) to 3 to 5 ml. of the sample. A gelatinous precipitate confirms alginates or de-esterified pectin.

IRISH MOSS. Add 2 to 3 drops of 0.5% methylene blue in water to 1 ml. of the sample solution. Precipitation of purple-stained fibers confirms Irish moss.

METHYLCELLULOSE. Mix 5 ml. of sample with 25 ml. of 95% alcohol and 2 to 3 drops of saturated sodium chloride. No precipitate confirms methylcellulose.

AGAR. Precipitate gum from 5 ml. of sample with alcohol and stain with tincture of iodine (3). Starch is also stained blue by this reagent.

STARCH. Add 1 to 2 drops of the iodine potassium iodide test solution to 1 ml. of sample. A blue or purple color confirms starch. Some samples of gum tragacanth may give a faint blue test here.

CARBOXYMETHYLCELLULOSE. Add 2 ml. of 1.0 *M* cupric sulfate to 3 to 5 ml. of sample solution. An opaque, slightly bluish, clotted precipitate confirms carboxymethylcellulose.

LOCUST BEAN GUM. Add 1 ml. of 4% borax to 3 to 5 ml. of gum solution. If mixture gelatinizes, locust bean gum is confirmed. Guar gum also forms a gel here.

KARAYA. Precipitate gum from 5 ml. of solution with alcohol and stain with ruthenium red (3). If sample swells considerably and is stained pink, karaya is confirmed.

GELATIN. Add 2 to 3 drops of gum solution to 2 ml. of saturated picric acid. A fine yellow precipitate confirms gelatin.

DISCUSSION

The proposed procedure has been tested with the gums listed in Table V. It was possible to identify the modified starches as starch products, because the solubility of all these materials is decreased by barium hydroxide and all give positive tests with the iodine-potassium iodide reagent. It is possible that not all the thickening agents or gums employed in food products at the present time have been included in this study. Cherry gum and quince seed gum, for example, have been suggested in the literature for use in foods. However, the proposed procedure includes all the gums that were available during the investigation.

The proposed scheme for identification of stabilizing and thickening agents is applicable only when they have not been mixed with other materials. To identify thickening agents in foods by this method it would first be necessary to separate them from the foods, but separation techniques might alter the reaction characteristics of the gums. Much work has been done on methods for the separation and detection of gums in particular foods, such as mayonnaise and French dressing (2, 7, 10, 11), soft curd cheese (1, 8, 10, 11, 13, 19), tomato products (10, 13), starchy foods (21), cacao products (13, 17, 23), ice cream and frozen desserts (9-11), canned chicken (10), and meat products (12). Additional references to methods for separating gums from foods may be found in reviews by Jacobs and Jaffe (15) and Mantell (16). The emphasis in most procedures has been on detection of the gums without identification. However, Wyler (24) has outlined methods for the detection and identification of locust (carob) bean gum, methylcellulose, carboxymethylcellulose, starch, pectin, and alginate in meat products. Thus identification tests-proposed in the present paper may be useful for the identification of gums separated from foods by methods already described in the literature. However, it is probable that many special techniques will be required for the analyses of particular combinations of foods and thickeners. A great deal more work must be done before it will be possible to identify all of the gums in the various foods in which they may be used.

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LETTERS TO THE EDITORS

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Specific Inhibition of Esterase in Ester-Hydrolysing Enzyme Systems

By mixing out several emulsifying agents having a fairly neutral saponifying effect upon emulsions of olive oil, monobutyrine, methylbutyrate and ethylpropanoate, the following emulsions were made. Gummi arabicum acts on one hand the emulsion of olive oil to a very remarkable degree, but exerts no effect upon the cleavage of monobutyrine. It causes on the other a very sharp inhibition of the saponification of such esters where it is substituted by lower alcohols. This inhibitory effect is found in several instances (depending upon the source of the active enzymes employed) to 100 per cent, and in no case was it less than 65 per cent. In the tests with olive oil, the activating effect of gummi arabicum proved to be dependent upon the stability of the emulsion, which on its part depends principally upon the degree of preparation and to a much less degree upon the absolute amount of gummi arabicum added. Thus, in several instances, where stable emulsions were obtained, the activating effect was either very small.

The concentration of the substrates employed in the enzyme tests (above) was in all cases 0.001 molar, while the concentration of oil was adjusted, according to its saponification value, to contain some amount of saponifiable fat. The amount of gummi arabicum added was in all cases half the amount by weight of the emulsifier. Commercial gummi arabicum was employed. The enzymes used were the glycerol extracts from pancreatin (Boehr. Davis and Co.), glycerol extracts from worker maggots of the honey bee of different ages, from organs of adult worker bees, and beef pancreas obtained in the usual way with the hydraulic press. To illustrate the results obtained, some examples are given in the accompanying tables. Concentration of substrate: 1 millimole per 10 ml. phosphate buffer 7.2 (Sørensen). The extracts of pancreas were prepared by grinding the biological materials with 10 ml. glycerol in a mortar (10 gm. glycerol per 1 gm. of substance) and leaving them overnight at 37° C. The undissolved part was centrifuged off and the extract diluted with buffer solution pH 7.2 (Sørensen) as described below. Values of additional cleavage are given for the blanks and given in ml. of 0.2% NaOH (Sørensen's titration). All tests were carried out at 37° C.

TABLE 1. ENZYME: 0.5 BEEF LIVER JUICE 1:5 (DILUTED WITH BUFFER SOLUTION) ADDED TO 20.5 ML. OF SUBSTRATE SOLUTION

Substrate	Time of action	Additional cleavage in 4 ml. solution	
		with gummi arabicum	without
Methylbutyrate	30 min.	0.30	0.60
	90 min.	0.45	1.25
	22 hr.	0.95	4.55
Ethylbutyrate	30 min.	0.20	0.30
	90 min.	0.35	0.65
	22 hr.	0.73	4.43
Ethylpropanoate	30 min.	0.05	0.40
	90 min.	0.25	0.95
	22 hr.	1.35	4.15

TABLE 2. ENZYME: 3 ML. GLYCEROL EXTRACT FROM WORKER MAGGOTS OF THE HONEY BEE, 5 DAYS OF AGE, 3:2 (DILUTED WITH BUFFER SOLUTION) ADDED TO 10 ML. OF SUBSTRATE SOLUTION

Substrate	Time of action	Additional cleavage in 5 ml. of solution	
		with gummi arabicum	without
Methylbutyrate	23 hr.	0.50	2.15
Ethylbutyrate	"	0.25	4.35
Ethylpropanoate	"	0.15	2.10

TABLE 3. ENZYME: 3 ML. GLYCEROL EXTRACT FROM WORKER MAGGOTS OF THE HONEY BEE, 8 DAYS OF AGE, 3:2 (DILUTED WITH BUFFER SOLUTION) ADDED TO 10 ML. OF SUBSTRATE SOLUTION

Substrate	Time of action	Additional cleavage in 5 ml. solution	
		with gummi arabicum	without
Methylbutyrate	23 hr.	0.45	1.40

TABLE 4. ENZYME: 3 ML. GLYCEROL EXTRACT FROM PANCREATIN 1:2 (DILUTED WITH BUFFER SOLUTION) ADDED TO 10 ML. OF SUBSTRATE SOLUTION

Substrate	Time of action	Additional cleavage in 5 ml. solution	
		with gummi arabicum	without
Methylbutyrate	23 hr.	2.10	7.40
Ethylbutyrate	23 "	0.85	8.00
Ethylpropanoate	23 "	3.40	3.35
Olive oil	23 "	1.75	0.85

It thus appears that there are at least two distinctly different enzymes (or enzyme systems) present in these glycerol extracts: (1) a lipase, hydrolysing esters of glycerol, which is not inhibited by gummi arabicum; and (2) an esterase, hydrolysing esters of lower alcohols than glycerol, which is inhibited by addition of gummi arabicum.

These substrates such as methylbutyrate and ethylbutyrate, usually recommended as standard substrates for the measurement of the activity of pancreatic lipase, seem, *de facto*, to undergo cleavage not by the lipase itself but by an esterase associated with the latter.

Further investigation into the nature of this inhibitory effect is proceeding.

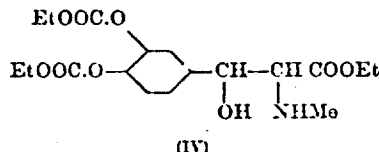
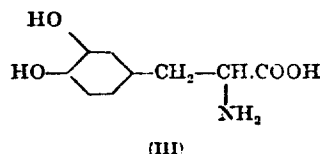
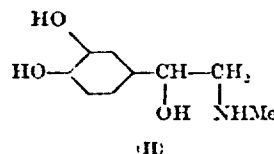
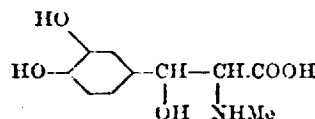
A detailed report will be given elsewhere.

Department of Biological and Colloidal Chemistry,
Hebrew University,
Jerusalem,
July 31.

P. J. FODOR

Adrenaline Carboxylic Acid
(N-Methyl-2-(3:4-dihydroxyphenyl)-serine)

THIS letter, unrecorded amino-acid (I) is of considerable pharmacological interest in view of its intermediate relationship to adrenaline (II) and to N-methyl-2-(3:4-dihydroxyphenyl)-serine (III). At the suggestion of Dr. H. Likaschko, we have consequently investigated its preparation.



The following synthesis has now been accomplished. Dicarboethoxy-protocatechuic aldehyde was condensed with sarcosine ethyl ester under the influence of sodium in ether to give ultimately N-methyl-2-(3:4-dicarboethoxydihydroxyphenyl)-serine ethyl ester (IV). Since the hydrochloride of this compound was a viscous syrup, it was converted to the oxalate, m.p. 147° (decomp.), which on recrystallization dissociated to give the monohydrated hydrogen oxalate, m.p. 157° (decomp.). Considerable difficulty was experienced in the attempted alkaline hydrolysis of salts of (IV). Hydrolysis was, however, smoothly effected in good yield with negligible oxidation by boiling with dilute acetic acid, and the amino acid (I), recrystallized from aqueous alcohol, formed cream-colored crystals, m.p. 23.2° (decomp.) (Found: C, 52.4; H, 5.5; N, 6.1 per cent. C₁₄H₁₃O₅N requires C, 52.0; H, 5.7; N, 6.2 per cent). No indication of the presence of more than one racemate was obtained.

Further work is required before the mechanism of the above condensation is elucidated, but certain interesting points have emerged. Rosenmund and Dornwaldt adduced evidence that the condensation of benzaldehyde with glycine ethyl ester involves the initial formation of a Schiff's base, CH₂[N(CH₂CH₂COOEt)]₂, which then condenses with a second molecule of the aldehyde to form PhCH(OH)CH(NCH₂CH₂COOEt)₂, from which the initial benzaldehyde residue is ultimately hydrolysed, giving the acid PhCH(OH)CH(NH₂)COOH. We find that our condensation does not succeed unless two molecules of aldehyde are used for each molecule of sarcosine ester. This suggests that the reaction may proceed through the stages CH₂[N(CH₂CH₂COOEt)]₂ → PhCH(OH)CH[N(CH₂CH₂COOEt)]₂ → PhCH(OH)CH(NHMe)COOEt, where R represents the 3:4-dicarboethoxydihydroxyphenyl group. It is noteworthy that we have been unable to condense various aldehydes with sarcosine ester, in spite of a wide variety of conditions employed, and the condensation appears to be critically influenced by the groups used to protect the two phenolic groups.

The examination of the amino-acid (I) is being undertaken in the Department of Pharmacology at Oxford. The description of our chemical work will appear elsewhere.

F. G. MANN
G. E. DALGLISH

University Chemical Laboratory,
Cambridge,
Aug. 6.

¹ Cf. Rosenmund and Dornwaldt, *Ber.*, 52, 1734 (1919).

THE ALLERGENIC PROPERTIES OF THE VEGETABLE GUMS

A CASE OF ASTHMA DUE TO TRAGACANTH

H. HAROLD GELFAND, M.D.

NEW YORK, N. Y.

REPORTS of sensitivity to various commercial gums have appeared increasingly during the past decade, with gum arabic (acacia) and karaya most often found to be the causative agents. The usual mode of entry is by inhalation, but ingestion and surface contact also have accounted for some cases. The symptoms include vasomotor rhinitis, bronchial asthma, urticaria, atopic dermatitis, angioneurotic edema, and gastrointestinal disturbances. In a large majority of cases sensitization occurs through occupational contact with the gums, and clinical symptoms arise only after a considerable period of exposure. In order to show the wide distribution of these allergens and the conditions under which they may be encountered, the reports thus far published will be briefly reviewed.

I. REVIEW OF THE LITERATURE

Acacia (Gum Arabic).—Experimental work with animals by Maytum and Magath¹¹ (1932) showed acacia to be mildly antigenic. It caused no reactions in rabbits, but varying degrees of anaphylaxis could be induced in about 63 per cent of the guinea pigs tested. Despite these findings, however, and the low nitrogen content of gum arabic, it has been shown to cause severe clinical symptoms in a large number of persons. The above-mentioned authors observed a patient under treatment for elephantiasis of the leg, who, following a Kondoleon operation, received 500 c.c. of a 6 per cent solution of acacia and 500 c.c. of physiologic saline solution intravenously. No untoward symptoms occurred at the time, but seven months later, when the therapeutic injection was repeated following a second operation, the patient developed nasal obstruction, lachrymation, loss of voice, coughing, and a suggestion of laryngeal stridor. These manifestations were mild and easily relieved by epinephrine. The patient had shown no previous signs of allergy, but her family history was positive.

Much more severe reactions to the intravenous injection of acacia were reported by Studdeford¹⁵ (1937). In three patients suffering from post-partum hemorrhage, infusions of acacia glucose solution produced acute constitutional reactions marked by cyanosis, dyspnea, tachycardia, and pulmonary edema. Two of the patients died, and autopsy upon

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one disclosed an extensive destructive lesion of the liver. Believing that impurities in the acacia preparations might have caused these untoward effects, the author tried fresh material from another supply house, but this produced similar results in three other patients, one of whom died.

Studdesford observed that recent experimental work has shown the likelihood of liver damage following the intravenous injection of acacia. It seriously disturbs the red blood cells, interfering with the normal gaseous interchange, increasing the tendency to rouleaux formation, and accelerating the sedimentation rate. Conglutination of red cells may occur, producing blockage followed by edema and hemorrhage.

Experiments on dogs by Hall, Gibson, and Weed⁷ (1940) showed that repeated intravenous injections of the gum damaged the carbohydrate and serum protein functions of the liver, as evidenced by changes in the glucose and galactose blood sugar tolerance curves and determinations of plasma proteins. However, no effect was observed on the hepatic cells, nor was there any blocking of the reticulo-endothelial system comparable to that produced by some colloidal substances.

Spielman and Baldwin¹⁴ (1933) described a case of acacia sensitivity in a plaster molder employed in a candy factory. He developed vasomotor rhinitis and bronchial asthma after working about six months in the plant. Direct tests produced a marked reaction to the factory dust containing crude acacia and also to purified acacia. Passive transfer tests likewise were positive.

Allergy to acacia among printers appears to be growing more and more frequent. The gum is used in solution with dextrin, alcohol, and water as a drying, or offset, spray for printed material. The spray fills the workrooms with a fine mist which the employees unavoidably inhale unless protected by a mask.

Feinberg and Schoenkerman⁵ (1940) reported a case of bronchial asthma in a printer which they attributed to this cause. The patient gave positive skin reactions to gum arabic and also to karaya. Following this, Bohner, Sheldon, and Trenis¹ (1941) published ten similar cases. All their patients reacted to gum arabic by direct tests, and passive transfer was positive whenever tried. These printers had been exposed to the acacia solution for periods of from two weeks to a year. The authors state that "the direct transfer tests were negative to Indian gum and tragacanth" ("Indian gum" probably referring to karaya).

King⁹ (1941) treated one case of bronchial asthma and another of vasomotor rhinitis in printers, both of whom were positive to acacia. These patients were of allergic constitution and reacted to other substances, such as orris root, egg white, house dust, and pollen, but clinical symptoms did not appear until they became sensitized by gum arabic in their occupation. Attempts at desensitization in one patient were unavailing while he remained in his job. King refers to the New

York State Department of Labor Bulletin for 1940,¹³ which also mentions a number of cases of rhinitis and asthma among printers, probably due to gum arabic.

According to our search through the literature, Levin¹⁰ (1939) appears to have been the first to record a case of sensitization to acacia in the printing trade. It probably is only in recent years that this type of offset spray has been brought into use.

Feinberg and Schoenkerman⁵ described the case of a furniture dealer with asthma of many years' standing, which probably was due to infection. Nevertheless, the patient gave a marked skin reaction to acacia, which proved to be an ingredient in some of his furniture preparations. Desensitization with the gum appeared to afford some temporary relief.

Karaya Gum.—Bullen³ (1934) was the first to report a case of allergy to karaya. His patient developed vasomotor rhinitis from contact with the gum in hair-waving lotion. Since that time such reports have become quite numerous. Feinberg⁴ (1935) observed bronchial asthma in a hairdresser from the same cause. Scratch tests with powdered karaya gum, sodium benzoate, tragacanth, acacia, and another brand of hair-waving fluid all were negative except that with karaya, which, he said, produced an "enormous reaction."

Bowen² (1939) reported five cases of urticaria due to karaya. One patient had associated respiratory symptoms. He gave details of one case in which the urticaria was accompanied by severe pruritus and some lesions of angioneurotic edema, frequently involving the joints. The edematous lesions were succeeded by patches of hyperpigmentation. This patient's symptoms were traced to karaya gum in Dr. Wernet's dental powder, used for holding her denture in place. She gave no family history of allergy.

Figley⁶ (1940) observed sixteen women with allergic symptoms, all of whom gave positive reactions to scratch tests with karaya. The family history was positive in all but two. The allergen was absorbed by ingestion and surface contact as well as by inhalation, and the chief manifestations were perennial rhinitis, asthma, atopic dermatitis, urticaria, and gastrointestinal distress. Wave lotions, laxatives, emulsified mineral oil, gelatins, diabetic foods, tooth pastes, and denture adhesive powders were the sources of sensitization.

In investigating the effect of Mucara, a laxative containing karaya, Ivy⁸ found that 7.8 per cent of eighty-nine test subjects who ingested this preparation complained of mild abdominal cramps or other discomfort for which he could not account. Figley suggested the possibility that these patients were slightly sensitive to karaya through having previously ingested it in some food or confection.

Feinberg and Schoenkerman⁵ analyzed ten cases of allergy to karaya resulting from contact with hair-waving lotions or powders. Respiratory

symptoms were the chief complaint in nine of the patients, one of whom had an associated dermatitis of the face and scalp. The tenth complained only of dermatitis of the face and neck. Cutaneous reactions to karaya were consistently positive in all the patients by direct test and also by passive transfer when this was performed. On being tested with other gums, in accordance with the authors' established routine, seven patients gave negative reactions, two reacted slightly to tragacanth alone, and one to acacia and tragacanth. The authors believed karaya to be the sole cause of clinical symptoms in four of the patients and a contributory factor in the remainder. In one patient with bronchial asthma, desensitization with karaya produced moderate improvement; in another, a change to tragacanth preparations proved beneficial.

The most common ingredients in hair-waving lotions are acacia, tragacanth, linseed gum, quince seed gum, karaya; boric acid, sodium, potassium, and ammonium carbonate; alcoholic keratin, coloe, petrolatum, cera-flux, glyco wax A, and paraflux. According to Figley, however, karaya has almost supplanted such gums as linseed and quince seed, and this fact may account for the growing number of allergic manifestations reported from contact with these preparations.

Gum Tragacanth.—Reports of sensitivity to tragacanth are extremely rare in the literature. One case has been described by Feinberg and Schoenkerman, in which a hay fever patient suffering from severe urticaria and eczematous dermatitis of the hands gave a marked reaction to tragacanth. The authors believed her cutaneous symptoms to be due to a hand lotion containing this gum but were unable to follow the case closely enough to obtain conclusive proof. We already have mentioned that three of their karaya-sensitive patients gave slight reactions also to tragacanth. They stated that they were unable to find any cases in the literature that incriminated tragacanth as an allergen, but "because of its close relation to karaya gum," they employed it in routine testing.

Owing to its comparative rarity, therefore, the following history* may be of particular interest. We believe it to be the first thoroughly authenticated case of clinical allergy caused by sensitivity to tragacanth.

B. T., white, female, aged 26 years, came to Gouverneur Hospital on July 7, 1941, to ask for desensitization to certain commercial gums which she believed to have caused her respiratory symptoms. She gave the following history:

In December, 1939, she went to work in the office of a New York gum factory. This firm imports various commercial gums from the Orient, and mills them on the upper floor of its office building. The material is passed from one floor to another through a chute which is not airtight,

*This case report was presented before the Associated Allergy Clinics of Greater New York at their fall meeting, Nov. 6, 1941. The presentation was made by Dr. Maury D. Sanger and discussed by the author.

and office workers as well as the millers are exposed to dust given off by the gums. The patient's desk was situated close to the chute, samples of the material were handled by her, and other samples were exposed in open containers on the office floor. Moreover, she frequently came in contact with the mill workers.

When the patient began to work in this place she was in perfect health and remained so for about a year. Then, in December, 1940, she became troubled with what she supposed to be a persistent head cold, with blocking of the nose, frequent sneezing, and profuse nasal discharge. She began to feel "run down" and decided to remain at home to recuperate. On a brief visit to the factory on New Year's Day, 1941, however, she apparently contracted a "fresh cold." After her return to work this also persisted, and by the end of February, fourteen months after her first contact with the gums, she began to cough and wheeze.

On March 3, 1941, fifteen months after first exposure, and coincident with the milling of an unusual amount of tragacanth, the patient was seized with a severe attack of asthma, lasting for eight hours, and finally was relieved by epinephrine.

From that time on, up to June 20, 1941, similar attacks occurred whenever she returned to her job after sick leave or vacations. All were relieved by epinephrine. After an extremely severe seizure on June 20, she resolved never to return to the factory. But, in order to prove that the gums were responsible for her trouble, she tried various temporary positions in other kinds of business. In the new surroundings, no untoward symptoms occurred.

Convinced now that she was allergic to the gums, and her return to the job being greatly desired, the patient came to our clinic to be desensitized.

Personal and family history, as given, were completely negative. Physical examination revealed no sign of infection in the nasopharynx or sinuses. X-ray examinations of the chest and sinuses also were negative.

Direct skin tests with the usual allergens produced no response, but when gum tragacanth, 100 units, was tried, a marked reaction occurred. Gum arabic, 1,000 units, also produced a strong reaction. Passive transfer tests with these two gums were strongly positive down to the 1:100 serum dilution. On the other hand, direct tests with karaya up to 5,000 units failed to provoke any response.

Immunization treatment with tragacanth was attempted with great caution for fear of untoward reactions, and the dosage increased at first only from 5 to 10 units for each successive treatment.

After receiving thirty injections, the patient believed herself to be immunized and decided to revisit the gum plant. Upon only a half-hour exposure, however, she experienced an ominous tightness in the chest.

TABLE I
OCCUPATIONAL ALLERGY IN A GUM FACTORY

CASE NO.	NAME	SEX	AGE (YR.)	TYPE OF WORK	TIME EMPLOYED	ALLERGIC SYMPTOMS	REACTION TO TESTS			REMARKS
							KARAYA	GUM ARABIC	TRAGACANTH	
Office Workers										
1	M. W.	M	63	Manager	30 yr.	None	Neg.	Neg.	Neg.	--
2	V. L.	M	25	Chemist	4 yr.	None	Neg.	Neg.	Neg.	--
3	M. K.	F	1	Clerk	1 yr.	Rhinitis, asthma	--	Mkd.	--	Left job
4	B. T.	F	27	Secretary	1 yr.	Rhinitis, asthma	Neg.	Mkd.	Mkd.	Left job
Factory Workers										
5	M. R.	M	42	Packer	11 yr.	Nasal clogging, dyspnea	Neg.	Mod.-Mkd.	Neg.	Asthma due to infection
6	G. S.	M	35	Miller	2½ mo.	Bronchial asthma	Neg.	Neg.	Neg.	--
7	L. O.	M	33	Miller	12 yr.	None	Neg.	Mod.-Mkd.	Neg.	--
8	J. P.	M	21	Elevator operator	1½ mo.	None	Neg.	Neg.	Neg.	--
9	J. C.	M	39	Laborer	15 yr.	None	Neg.	Neg.	Neg.	--
10	E. W.	M	38	Shipping clerk	4 yr.	None	Mod.	Mod.-Mkd.	Neg.	--
11	A. O.	M	5	Foreman	5 yr.	Nasal clogging, sneezing	Neg.	Mod.-Mkd.	Mod.	--
12	J. S.	M	22	Miller	1½ yr.	Sneezing, dyspnea	Mod.	Mod.-Mkd.	Mkd.	--
Summary										
	NUMBER EXAMINED	NUMBER AFFECTED	NUMBER NOT AFFECTED	CAUSE OF SYMPTOMS		POSITIVE SKIN REACTIONS				
				GUMS	INFECTION	KARAYA	GUM ARABIC	TRAGACANTH		
Total	12	6	6	5	1	2	7	3		
Office workers	4	2	2	2	0	0	2	1		
Factory workers	8	4	4	3	1	2	5	2		

On reaching home she was seized with an exceedingly severe attack of bronchial asthma. A number of injections of epinephrine were required before the paroxysms subsided, and she remained ill for a week. This experience caused the patient to abandon all hope of returning to her job, and she also discontinued treatment.

II. SENSITIZATION TO GUMS AS AN OCCUPATIONAL RISK

The striking character of the foregoing case and the excellent field for research provided by a factory solely concerned with the handling and processing of allergenic gums led us to study the incidence of sensitivity among the other employees.

We found that the firm had been in business for more than a century, and during that time certain employees in the mill (formerly in a separate building) had had symptoms of vasomotor rhinitis and severe bronchial asthma after contact with tragacanth, gum arabic, and karaya. In most cases, the affected workers had to leave their occupation, but some were able to carry on, and, in time, appear to have become, so to speak, immune.

In recent years the milling has been done in the same building with the executive offices, and we learned that another female office worker, previous to our survey, had been obliged to give up her work on account of nasal symptoms and bronchial asthma, coming on about a year after first exposure. Her physician had found her to be sensitive to gum arabic.

In order to gain more information as to the sensitizing properties of the gums and their antigenic relationships, we carried out a study among both the office and mill workers. We were unable to examine the entire personnel, but those who were willing to undergo tests made up a good cross-section.

No further complaints were discovered among the office force, but investigation of the millers revealed a somewhat different picture. Most of the men had been in the mill for a number of years under conditions of massive exposure, and the factor of mechanical irritation as well as prolonged contact was involved. Nevertheless, many of them had escaped untoward symptoms. Table I shows the results of our examinations thus far.

It will be noted that 50 per cent of all the workers examined had respiratory symptoms of an allergic nature. Among the four office employees, one* was sensitive to gum arabic and another to gum arabic and tragacanth but not to karaya. These workers had to leave their jobs. Of the eight mill workers, three had nasal clogging and sneezing (with or without constriction of the chest) when in contact with certain gums, and these three were found sensitive to the materials by direct

*This refers to M. K., who had left the plant previous to our survey. However, since we were able to obtain her history, it has been included.

skin tests. One had asthma traceable to infection in the sinuses and unrelated to his occupational contacts.

Comment.—In view of the contradictory findings in a few of these cases, the question arises—how many of the men who gave positive skin tests, but denied having symptoms, were concealing their complaints for fear of endangering their jobs? With one or two of them we had a strong impression that this was the case. Nevertheless, the fact remains that a certain number of employees definitely remained free from untoward symptoms and had no signs of clinical pathology discoverable by physical or roentgen examination or by skin tests. Those who did acknowledge allergic symptoms, with one exception, also gave positive skin reactions to one or more of the gums. Hence, it is clear that irritation and prolonged contact with tragacanth, gum arabic, and karaya affect some persons but fail to affect others working under the same conditions. We must conclude that the individuals who become sensitized have an inherent predisposition to allergy, which probably is hereditary, although no family history of hypersensitiveness could be elicited in any of these cases.

The case of B. T. is the most clear-cut of any that came under our observation and the most enlightening. First, it demonstrated the fact that gum tragacanth as well as gum arabic is a sensitizing agent capable of producing severe respiratory symptoms in predisposed individuals. Second, the patient was found, by both direct and passive transfer tests, to be sensitive to gum arabic and tragacanth but not to karaya, despite the fact that the antigenicity of karaya has been so well established. This tends to disprove the general assumption that all these gums are closely related. Third, it was shown that a period of time was required after first contact to bring forth the acute sensitivity. Fourth, since the treatments though limited were supplemented by long absence from exposure and yet failed to confer any degree of immunity, desensitization probably would be unavailing.

III. ANTIGENIC RELATIONSHIP OF THE GUMS

The general impression has been that the three gums under consideration are closely related both antigenically and botanically, but few observers appear to have carried out in vitro and in vivo neutralization experiments with the sera of patients known to be clinically sensitive to them. Since our clinical findings in the cases of B. T. and A. O. seemed to disprove the theory of a common antigen in karaya and the other two gums, we decided to make such studies in order to determine the antigenic relationship of tragacanth and gum arabic.

Sera were obtained from all the workers found to be sensitive, and all except one gave a positive passive transfer. For the neutralization studies, however, only one serum, that of B. T., remained suitable for study. A few hemolyzed, and the patients refused to give more blood. In one case the Wassermann was doubtful.

Following is the result of our studies with B. T. serum:

Exhaustion-Site Studies (Figs. 1 and 2).—

Method: Duplicate sites in nonatopic recipients were passively sensitized by injecting 0.1 c.c. of B. T. serum, which was clinically sensitive to gum tragacanth and gum arabic. The next day, when the reagins were fixed in the tissues, the first site (above) was tested with

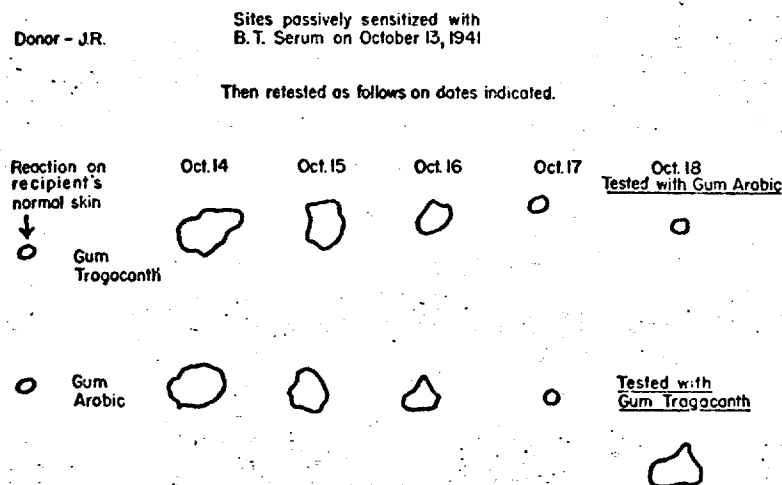


Fig. 1.

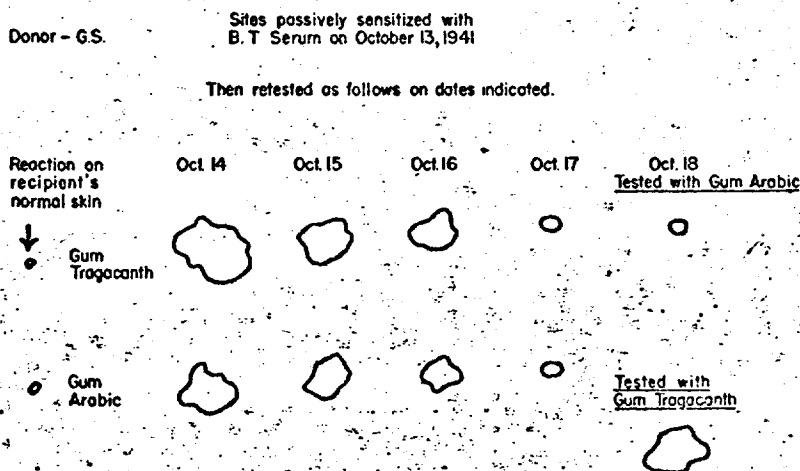


Fig. 2.

gum tragacanth and then retested daily until no reaction occurred. The following day the site was tested with gum arabic. The second site was desensitized in the same way to gum arabic and then tested with tragacanth.

Results: In both donors the reagins to gum arabic were completely neutralized by tragacanth, but gum arabic failed to neutralize the reagins to tragacanth.

In Vitro Neutralization Studies (Figs. 3 and 4).—

Method: Having previously determined that the immediate and forty-eight-hour tests showed neutralization at 500 to 700 units,* we prepared in the test tube under sterile precautions a mixture of two parts extract (0.2 c.c. of 5,000 units, representing 1,000 units) to one

Neutralization Studies on Tragacanth and Arabic Gums

Serum B.T.
Donor—J.R.

24 hours' incubation
in vitro of two parts
Tragacanth and one
part B.T. Serum

October 15, 1941

Mixture introduced
in Site of donor
(1/10 cc.)
Immediate Reaction

October 16, 1941

Site tested with
Gum Arabic

24 hours' incubation
in vitro of two parts
Arabic and one part
B.T. Serum

Mixture introduced
in Site of donor
(1/10 cc.)
Immediate Reaction

Site tested with
Gum Tragacanth

Fig. 3.

Neutralization Studies on Tragacanth and Arabic Gums

Serum B.T.
Donor—G.S.

24 hours' incubation
in vitro of two parts
Tragacanth and one
part B.T. Serum

October 15, 1941

Mixture introduced
in Site of donor
(1/10 cc.)
Immediate Reaction

October 16, 1941

Site tested with
Gum Arabic

24 hours' incubation
in vitro of two parts
Arabic and one part
B.T. Serum

Mixture introduced
in Site of donor
(1/10 cc.)
Immediate Reaction

Site tested with
Gum Tragacanth

Fig. 4.

part serum (0.1 c.c.) for each gum, tragacanth and arabic. After being allowed to stand in the icebox for twenty-four hours, each mixture was injected in 0.1 c.c. amounts into duplicate sites on nonatopic subjects.

*This was done by mixing in the test tube increasing amounts of antigen with a fixed amount of sensitive serum, allowing it to incubate in the icebox, and then testing test amounts into a nonatopic donor. The immediate reaction was observed, and 48 hours later each site was tested with 1,000 units of the gum antigen, and the neutralization point was noted.

Twenty-four hours later the sites injected with serum neutralized to tragacanth were tested with gum arabic, and vice versa. Duplicate control sites, having been prepared with two parts physiologic saline solution and one part serum, also were tested with each gum.

Results: Here, too, we found that tragacanth neutralized the reagents to itself as well as to gum arabic, but arabic, while neutralizing its own reagents, failed to neutralize the reagents to tragacanth.

Comment.—The direct skin tests in the case of B. T. suggested a probable antigenic relationship between tragacanth and arabic, but none between either of these gums and karaya. The serologic studies both in vivo and in vitro confirmed the relationship between the two gums to which the patient was clinically sensitive, since it was shown that gum tragacanth completely neutralized the reagents to gum arabic, but that gum arabic failed to neutralize all the reagents to tragacanth. It was noted that tragacanth and arabic extracts were similar in nitrogen content but that direct skin tests on this patient elicited a greater reaction to tragacanth. The impression was thus gained that tragacanth extract either contains an antigen not present in gum arabic or that it is richer than gum arabic in atopic excitant content.

IV. NATURE AND DERIVATION OF ALLERGENIC GUMS

This is a subject on which the information from various authoritative sources long has been indefinite and conflicting. Medical knowledge is therefore in a state of confusion, and it is small wonder that statements appearing in the literature show many contradictions.

We have consulted different authorities and have obtained the following information from the United States Dispensatory for 1937:

The term gum, often loosely applied to the resinous exudate of plants, refers more correctly to exudates which contain a carbohydrate that is capable of forming with water mucilaginous mixtures. The belief that gums differ from resins in being soluble in water and insoluble in alcohol is incorrect. Some gums do not actually dissolve in water but simply swell into viscid mixtures with it, and some gums are soluble in alcohol.

The gums that are most often considered identical, or whose names are frequently confused with one another, are described as follows:

Bassora Gum (Caramania Gum, or Hog Gum): Bassora gum is a collective term for a group of high-colored gums somewhat resembling tragacanth. The soluble part is arabin and is said to constitute about 11.2 per cent. The insoluble part consists of bassorin, with a small proportion of saline substances. The gum is used as an adulterant for acacia and can be distinguished by its insolubility in water. Its botanical origin is doubtful.

Karaya Gum: Karaya gum is known as Sterculia gum or Indian tragacanth. It is derived from *Sterculia urens* Roxb. (Fam. Sterculiaceae), of Asia, and possibly other species of *Sterculia*. It is found in irregularly shaped, pinkish brown or light

brown pieces and has an astringent odor. When boiled with 5 per cent potassium hydroxide solution, it shows only a slight tinge of brown, whereas tragacanth thus boiled becomes a bright yellow and gives a stringy precipitate. Tragacanth is more mucilaginous than karaya, and karaya has greater acidity (due to acetic acid) and dissolves more readily in cold water. Nevertheless, powdered karaya often is used as a substitute for powdered tragacanth and loosely called tragacanth.

India Gum (Ghatti Gum): The term India gum has been applied to many different substances, including Bassora gum, Sterculia gum, and Ghatti gum (*Gummi indicum*). The British Pharmacopoeia in 1914 recognized Ghatti gum as India gum. It is derived from different species of the Indian tree *Anogeissus latifolia* (Fam. Combretaceae). It is used in pharmacy for the same purposes as acacia. Its mucilage is more viscid but less adhesive than acacia and is usually employed in connection with tumeric, with which there is some specific combination.

Gum Arabic (Acacia): Gum arabic comes from the stems and branches of *Acacia senegal* Willdenow (Fam. Leguminosae), or some other African species of *Acacia*. It is whitish yellow or light amber in color and is insoluble in alcohol but almost completely soluble in water, the solution being acid. It is composed essentially of the calcium salt of arabin, or arabic acid. The gum is an effective demulcent and is extensively used in drugs, pills, and lozenges.

Gum arabic often is adulterated with Mesquite gum, from a Mexican plant, *Prosopis juliflora* (Fam. Leguminosae).

Tragacanth Gum: This substance is an exudation from *Astragalus gummifer* Labillardiere (Fam. Leguminosae) and other Asiatic species of *Astragalus*. In the past there has been much doubt as to its botanical source, but it now is known to be of the above genus and family. It is entirely insoluble in alcohol and seems to be composed of two different constituents, one being soluble in water and resembling gum arabic, and the other swelling in water but not dissolving. The soluble part, which is much the greater, is said by A. G. Norman (1931) to consist of uronic acid and arabinose in about equal proportion, which compose 94 per cent, plus small amounts of cellulose, starch, and protein substances. The insoluble part is mostly bassorin.

Tragacanth is sometimes adulterated with Sterculia (karaya) and Ghatti gum but more frequently with acacia. The difference between true tragacanth and Sterculia can be proved by a distillation test with water and determination of the acidity, which is greater in Sterculia.

According to Chemical Abstracts (1939), tragacanth contains enough starch to give a blue color with iodine solution, but acacia contains no starch. The methoxyl (CH_3O —) indexes of the three gums under consideration are: acacia, 0.12.4; tragacanth, 18.6-38; karaya (Sterculia), 0.

Figley⁶ quotes Norman (1929) to the effect that no essential difference exists between gums and hemicellulose; in both, hexose and pentose are linked with uronic acid. He also quotes Solis-Cohen, who states that arabin, bassorin, and cerasin are the proximate principles of gums, and that gums are chiefly pentosans.

Bohner, Sheldon, and Trenis¹ say that acacia is classed chemically as an inert colloid and is considered to be a polysaccharide member of the carbohydrate family, related polysaccharides being glycogen, dextrin, and starch. All of these are amorphous, odorless, and translucent, and on hydrolysis yield one or more sugars, usually pentoses and hexoses.

In discussing Bullen's paper,³ Baldwin said the fact that gums are listed as polysaccharides is misleading, because in the case of acacia, 0.5 per cent of total nitrogen is present and it also gives a positive biuret test. In an analysis of three samples purchased in the open market, Bohner and his associates found an average of 0.48 per cent of nitrogen and a positive biuret test. They note that Uhlenhuth and Remy¹⁶ found 0.3 per cent of nitrogen in samples of purified acacia.

Figley⁶ stated that the nitrogen content of karaya is only 0.1 per cent.

V. SOURCES OF CONTACT WITH ALLERGENIC GUMS

We have compiled the following list from data gathered by many of the authors quoted in this paper and from other available sources. Since we know that the various gums frequently are substituted for one another and adulterated by one another and by other gums, no attempt at separate classifications has been made:

- Adhesive pastes
- Artificial flowers
- Body and drier in lithograph inks
- Candy (e.g., gumdrops and jellies)
- Cement
- Cheese
- Cigar manufacture
- Coating for special thread
- Custards (e.g., in factory-made pasteries, etc.)
- Denture adhesive powders (Dr. Wernet's, Dent-A-Firm, Stix)
- Diabetic foods (e.g., soy bean and almond wafers)
- Emulsions (e.g., mineral oil, cod-liver oil, turpentine, almond, and flavor emulsions)
- Fireworks
- Furniture polishes
- Gelatines
- Glues
- Ice cream mixes
- Insecticides
- Laxatives (e.g., Imbicoll, Mucara, Squibb's Petroleum and Agar)
- Linoleum and oil cloth
- Lotions (cosmetic, for hand care, hair-waving, etc.)
- Luster for textiles
- Match manufacturing
- Metal polish manufacturing
- Mucilages
- Paints
- Pills
- Porcelain and pottery manufacture
- Printing ink manufacture
- Process engraving
- Salad dressings (factory made)
- Shoe polishes
- Sizing of paper and textiles
- Sprays (offset in printing trade)

Search (special)
Suppositories
Toothpastes (Listerine and Lactora)
Textile printing
Vaginal jellies
Varnishes
Water colors (transparent)

No doubt these gums also occur in many other preparations in which their presence has not yet been disclosed, and the sources of contact probably are even more widespread than the above list would indicate.

SUMMARY

1. The published reports of sensitivity to vegetable gums have been reviewed. These reveal that the chief sources of sensitization are karaya gum in hair-waving lotions and acacia in the offset sprays used in the printing trade. Although the allergens may enter the system by ingestion, injection, or surface contact, inhalation is the most common route, and respiratory symptoms predominate.

2. The first well-established case of sensitization to tragacanth is reported. This case led to a study of the incidence of gum sensitivity among the workers in a gum factory, which has brought forth the following facts: (a) that gum sensitization is an occupational risk for predisposed persons; (b) that a period of time (usually about a year) is required after first exposure for the development of acute symptoms; (c) that tragacanth is a powerful allergen capable of causing extremely severe reactions; (d) that desensitization with allergenic gums is difficult, if not impossible; (e) that some workers who become sensitized may afterward spontaneously develop tolerance.

3. The antigenic relationship between gum arabic and tragacanth has been studied by means of in vivo and in vitro experiments with sensitive sera. It was found that tragacanth is able to neutralize all the reagins to gum arabic but that arabic only partially neutralizes the reagins to tragacanth. Thus it appears that, while an antigenic relationship probably exists between these gums, and although the nitrogen content may be the same in both extracts, tragacanth must either contain an antigen not present in arabic or its extract must be richer in excitant content. The relationship between karaya and the other gums was not studied.

4. Consultation of various authorities on the botanical origin of the three gums revealed that gum arabic (*Acacia senegal*) and tragacanth (*Astragalus gummifer*), although differing in genus and species, are members of the same family (Leguminosae). Karaya, on the other hand, belongs to an entirely different family (Sterculiaceae). This fact may have some bearing on the antigenic relationship of the gums as brought out in our studies.

5. The known sources of possible contact with allergenic gums have been listed.

6. In conclusion, a review of the entire subject of gum sensitization shows it to be an increasing risk for predisposed individuals, especially through occupational contact, as demonstrated in hairdressers, confectioners, printers, employees of gum plants, and other workers who are subject to prolonged exposure.

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DISCUSSION

DR. KARL D. FIGLEY, Toledo, O.—It may be of interest to know that since I read Dr. Gelfand's paper I reviewed twenty-six cases of karaya gum sensitivity. These were all women who contacted karaya gum chiefly by inhalation of wave-set material containing the gum. Two of these persons were beauty parlor operators and the remainder were housewives or unmarried women. These twenty-six persons were all tested with acacia and tragacanth. None of them reacted to tragacanth; four, while giving negative scratch tests to acacia, did give 1 or 2 plus to acacia on intradermal testing. I think the reason none of my patients were definitely sensitized to acacia or tragacanth, is that in all probability they never were exposed to these substances by inhalation. Therefore, they obviously would not be sensitized.

It is interesting that Dr. Gelfand's twelve patients studied were presumably equally exposed by inhalation to the three gums; that the greatest number (six) reacted to gum arabic; that tragacanth was next with three reactors; and karaya last with two reactors. May one assume from this that arabic is the most potent allergen of the three?

DR. HOWARD OSGOOD, Buffalo, N. Y.—I have been testing routinely for some time with five different gums but have done no passive transfer studies. Just before leaving for this meeting, I pulled out at random the records of 100 allergic patients and summarized the results of the tests with these gums. I thought it might be of interest in connection with the present paper.

The gums used were acacia, tragacanth, quince seed, karaya, and ghatti. The latter is sometimes substituted for karaya in what is known as India gum. The concentrated extracts were 1:33 or 1:100 of the dry gum in Coea solution, depending on the ease of Seitz filtration. A 1:10 dilution was made up from the concentrated extract, and tests were carried out in each patient with both strengths.

Of the 100 patients tested, there were only two who failed to give skin reactions in some degree (slight, moderate, or marked) to one or more of the gums, in weak or strong dilution. There were twelve patients who reacted to one gum only, twenty-two who reacted to two only, twenty-two to three gums, fourteen to four, and there were twenty-eight patients who reacted in some degree to all five gums.

To determine the relative skin-exciting properties of the different gums, or conversely the relative skin reactivity of this group of 100 patients to the different gums, I have tabulated the maximum reaction given by each patient to each gum, without indicating to which strength (concentration or 1:10 dilution) the maximum reaction occurred. For example, to acacia thirty-four patients gave only a slight reaction to either dilution, thirty-three gave a moderate reaction, and twelve gave a marked reaction to one or the other dilutions. Ghatti gum gave the most numerous reactions, ninety-three patients reacting to some degree. Karaya gum with only twenty-five patients reacting, gave the fewest.

TABLE I
MAXIMUM DEGREE OF SKIN REACTIONS TO EACH GUM

	NUMBER OF PATIENTS GIVING				MODERATE OR MARKED
	SLIGHT ONLY	MODERATE ONLY	MARKED	TOTAL	
Acacia	34	33	12	79	45
Ghatti	42	36	15	93	51
Karaya	25	6	5	36	11
Tragacanth	26	12	10	48	22
Quince seed	42	16	4	62	20

I tried to see what cross reactions there might be to these five gums and have tabulated the frequency of associated reactions in single patients. Moderate and marked reactions only were considered. Eighteen gave moderate or marked skin reactions to acacia and ghatti gums and to no others, this being the most frequent association. The number of other patients giving reactions to two gums only showed the following associations: acacia-tragacanth, 2; acacia-quince, 2; ghatti-karaya, 2.

platti-quince, 1. The number of patients showing reactions to three gums only gave the following associations: acacia-ghatti-quince, 9; acacia-ghatti-tragacanth, 5; ghatti-tragacanth-quince, 1. Eight patients gave moderate or marked reactions to four or to all five gums. It will be seen that the most frequent association of moderate or marked reactions occurred with acacia, ghatti, and quince seed.

I am presenting this statistical summary for what it may be worth and draw no definite conclusions. I am not ready to state that these positive skin reactions indicate clinical sensitivity to gums, although in a small number of these 100 patients this was definitely the case.

My own feeling is that in the dilutions used, the gum solutions may be intrinsically slightly irritating, thus explaining the numerous slight and moderate reactions. Typical marked reactions occurred in forty-six patients, most frequently to ghatti, acacia, and tragacanth. My second opinion is that there may be common allergens in some of the gums.

I have not delved into botanical relationships, as has Dr. Gelfand, but from his results, and from my meager observations, it would seem that further work with these gums along the lines laid out by Dr. Gelfand would be well worth while.

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(Continued)

THE VEGETABLE GUMS BY INGESTION IN THE ETIOLOGY OF ALLERGIC DISORDERS*†

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THE vegetable gums as causative factors in allergic disturbances, by means of inhalation, surface contact, and injection, have been recorded by various investigators¹⁻⁷ including the present writer.¹¹ The present paper stresses the importance of these gums as agents that cause allergic symptoms by ingestion.

Ivy and Isaacs' report⁸ of the effect of the laxative Mucara which contains karaya should be mentioned here. In a group of eighty-nine test subjects, 7.8 per cent complained of mild abdominal cramps or other discomfort after the ingestion of this laxative.

Figley⁹ observed sixteen women with allergic symptoms, all of whom gave positive reactions to scratch tests with karaya. The family histories for allergy were positive in all but two of these subjects. The allergens had been absorbed by ingestion and by surface contact, as well as by inhalation. The chief manifestations were perennial rhinitis, asthma, atopic dermatitis, urticaria, and gastrointestinal distress. Wave lotions, laxatives, emulsified mineral oil, gelatins, diabetic foods, tooth pastes, and denture adhesive powders were the sources of sensitization.

The first case report of an allergic reaction by ingestion, to drug tablets containing the vegetable gums, was presented by Brown and Crepea.¹⁰ This was a white man who manifested symptoms of asthma and generalized urticaria from the ingestion of Pyribenzamine tablets which contain tragacanth. Skin tests, passive transfer, and clinical trial established this vegetable gum as the cause.

Further proof of the role of the vegetable gums, by ingestion, in the causation of allergic disorders will form the basis of the present paper. In Table I is recorded a series of ten subjects suffering from allergic disorders caused by the ingestion of foods which contained karaya, tragacanth, or arabic. Direct

*From the Department of Allergy, the Gouverneur Hospital.

†Read before the Fifth Annual Meeting of the American Academy of Allergy, Atlantic City, N. J., Dec. 6-8, 1948.

TABLE I. ALLERGIC DISORDERS IN TEN SUBJECTS RESULTING FROM INGESTION OF FOODS CONTAINING THE VEGETABLE GUMS

CASE AND SUBJECT	AGE AND SEX	GUM-CONTAINING FOODS CAUSING SYMPTOMS	CLINICAL MANIFESTATIONS OF ALLERGY	SKIN-REACTING GUMS	OTHER ALLERGENIC SENSITIVITIES OF IMPORTANCE
1. R. L.	22 F.	Cream cheese, fillings in candies, marshmallows	Vasomotor rhinitis, gastro-intestinal symptoms, epigastric distress, nausea, belching, flatulence	Karaya Tragacanth* Arabic	Dust
2. L. K.	11 M.	Commercially prepared pie crust mixtures, marshmallows, certain brands wheat cakes, certain brands cream cheese, commercially prepared cake icing, gelatine	Vasomotor rhinitis, gastro-intestinal symptoms, urticaria, allergic cough	Arabic Karaya	Dust, animal epithelia, orris root, cotton, silk, chocolate, fish, paprika, mushroom
3. R. S.	35 M.	Commercially prepared white sauce, commercially prepared mustard, processed cheddar cheese, processed Swiss cheese, commercially prepared potato salad	Urticaria, angioedema	Tragacanth Arabic	Dust
4. R. B. A.	37 F.	Commercially prepared cake icing, commercially prepared whipped cream, ice cream mixtures	Vasomotor rhinitis	Karaya Arabic	Dust
5. J. G.	29 M.	Commercially prepared cake icing, fillings in candies, cream cheese	Gastrointestinal allergy: epigastric distress, belching, flatulence, diarrhea	Karaya Tragacanth Arabic	Dust, animal epithelia, tobacco, timothy, ragweed, mushroom, spinach, banana
6. A. S.	13 M.	Certain brands of tooth paste	Vasomotor rhinitis: clogged nose, running nose, sneezing	Arabic Karaya Tragacanth	Dust
7. M. G.	45 M.	Certain brands of gum	Bronchial asthma	Tragacanth Arabic	Trees, grasses, ragweed, dust
8. G. C.	35 M.	Gelatines, commercial whipped cream in "charlotte russe," fillings in candies	Urticaria, angioedema, itchy gums	Karaya Tragacanth Arabic	Dust, trees, ragweed, animal epithelia
9. B. D.	16 F.	Ice cream mixture, frozen custard	Vasomotor rhinitis, bronchial asthma	Karaya Tragacanth Arabic	Dust, trees, grasses, ragweed, animal epithelia
10. L. D.	55 M.	Commercially prepared salad dressings, shrimp sauce and other	Vasomotor rhinitis: nasal congestion, rhinorrhea, clogged nose, sneezing	Karaya Tragacanth Arabic	Trees, grasses, dust, chocolate, orange

SUMMARY OF TABLE I

TOTAL NO. OF SUBJECTS	AGE RANGE	NUMBER OF INDIVIDUAL FOODS CONTAINING THE GUMS	TOTAL NUMBER OF ALLERGIC MANIFESTATIONS	NUMBER GIVING POSITIVE SKIN REACTIONS TO THE GUMS		
				KARAYA	ARABIC	TRAGA- CANTH
10 7 males 3 fe- males	11-55 years	19	7	8	10	8
		Cake icing (com- mercially pre- pared) Cheddar cheese (processed) Cream cheese Fillings in candies Frozen custard Gelatin Gum (certain brands) Ice cream mix- tures Gelatin Marshmallows Mustard (com- mercially pre- pared) Pie crust (com- mercially pre- pared) Potato salad (commercially prepared) Salad dressings (commercially prepared) Shrimp sauce (commercially prepared) Swiss cheese (processed) Tooth paste (certain brands) Wheat cakes (certain brands) Whipped cream (commercially prepared) White sauce (commercially prepared)	Bronchial asthma Allergic cough Angioedema Gastrointestinal allergy Urticaria Vasomotor rhini- tis Itchy gums			

skin tests with the gums, clinical trial and elimination, and positive serologic findings confirmed these suspicious allergens as the causative agents. The allergic manifestations recorded in Table I were not only gastrointestinal reactions but consisted also of many other systemic reactions of allergy such as bronchial asthma, generalized urticaria, vasomotor rhinitis, and so forth. Elimination of the suspected irritant foods obviated the symptoms. Clinical trial repeatedly reproduced these symptoms.

SEROLOGIC STUDIES

Four sera in this group were found to contain abundant antibodies, and thus were suitable for passive transfer and neutralization studies. Results of these studies are shown in Tables II and III, respectively. The dominant gum antigens in these four subjects were: tragacanth and karaya in J. G. and B. D., and arabic in A. S. and L. D.

TABLE II. PASSIVE TRANSFER TESTS IN SERIAL DILUTIONS ON THE FOLLOWING GUM-SENSITIVE SERA

		These sites were tested 48 hrs. later with 1/40 to 1/20 c.c. of:		
		1% gum karaya	1% gum tragacanth	1% gum arabic
1. J. G. serum.—Test sites of J. G. serum in serial dilutions were introduced into the back of a nonallergic donor		Reactions		
Concentrate		marked	marked	marked
1 : 10		moderate	moderate	slight to moderate
1 : 100		slight	slight	negative
2. B. D. serum introduced in the same manner				
Concentrate		marked	marked	marked
1 : 10		slight	moderate to marked	slight
1 : 100		negative	slight	negative
3. A. S. serum introduced in the same manner				
Concentrate		moderate	moderate	marked
1 : 10		slight	slight	moderate
1 : 100		negative	negative	negative
4. L. D. serum introduced in the same manner				
Concentrate		moderate	moderate	marked
1 : 10		slight	slight to moderate	marked
1 : 100		slight	slight	slight

As can be seen from Table II, J. G., B. D., L. D., and A. S. showed strongly positive sera which transferred passively on a nonallergic donor.

From the neutralization studies, the following concrete conclusions may be drawn with respect to cross reactions between (1) tragacanth and karaya and (2) between tragacanth and arabic:

(1) In three of the four sera studied the evidence is presented that tragacanth neutralizes karaya. In one serum (J. G.) in which the addition of tragacanth to the serum failed to neutralize karaya, karaya was an unusual highly dominant clinical antigen in this subject.

(2) It was previously shown by the writer¹¹ that tragacanth neutralizes arabic, but arabic will not neutralize tragacanth. Apparently this pertains only in a serum in which tragacanth is the more dominant antigen. In the present study upon these highly gum-sensitive sera, it is evident that the clinical sensitivity dominance of either of these two antigens determines the capacity of one of them either to neutralize or not to neutralize its mate.

INGESTION OF THE POWDERED GUMS IN SENSITIVE SUBJECTS

Experimental Studies.—The vegetable gums, karaya, tragacanth, and arabic, were compounded in powder form and 300 mg. of the mixed gums were calculated by weight to be contained in each powder. 4 subjects (cases 5, 6, 7, 8).

9, and 10) were fed powders containing these gums, and one subject (Case 1) was fed candies containing gum fillings. Results of these experiments were observed and recorded as follows:

*Ingestion Experiment: Case 1 (R. L.)** had manifested the clinical forms of allergy as vasomotor rhinitis and gastrointestinal disturbance from the ingestion of foods containing vegetable gums. A brand of cream cheese, fillings in candies, and marshmallows were found to be the incriminating sources of allergic symptoms.

TABLE III. NEUTRALIZATION TESTS

		Three sites of .1 c.c. of each mixture for each serum were introduced into the skin of a nonallergic donor. After 48 hours these sites were tested with 1/40 to 1/20 c.c. of:		
		1% gum karaya	1% gum tragacanth	1% gum arabic
A. Equal parts of J. G. serum and the three gum antigens of 1% concentration were mixed separately in vitro as well as equal parts of J. G. serum and saline as a control:*		Reactions		
J. G. serum + karaya		slight	marked	moderate
J. G. serum + tragacanth		marked	moderate	moderate
J. G. serum + arabic		moderate	marked	slight
B. Same method of mixture, using B. D. serum as follows:				
B. D. serum + karaya		very slight	marked	moderate
B. D. serum + tragacanth		slight	moderate	negative
B. D. serum + arabic		moderate	marked	negative
C. Same method of mixture, using A. S. serum as follows:				
A. S. serum + karaya		very slight	moderate	marked
A. S. serum + tragacanth		negative	very slight	marked
A. S. serum + arabic		slight	slight	slight to negative
D. Same method of mixture, using L. D. serum as follows:				
L. D. serum + karaya		very slight	moderate	moderate to marked
L. D. serum + tragacanth		slight	very slight	marked
L. D. serum + arabic		slight	moderate	slight

*Serum plus saline controls were carried out in each of the four sera; the results resembled closely the serum dilution passive transfer tests given in Table II.

The following ingestion experiment was carried out in this subject: (This patient's favorite candies had been declared, by Dr. Pacini of Universal Colloid Company, to contain the vegetable gums.) For the experiment, Dr. Pacini arranged with a candy manufacturer to make up candies in two distinct layers. The top layer contained the vegetable gums, the bottom layer contained no vegetable gums. The patient, of course, had no knowledge of the contents of the candies. The layers were interchanged and fed to the patient at various intervals. Onset of the gastrointestinal symptoms in the form of belching, nausea, epigastric distress, and flatulence were invariably reported after ingestion of the candies containing vegetable gum fillings.

*Ingestion Experiment: Case 5 (J. G.)** had been found markedly sensitive to karaya, tragacanth, and arabic. Trial and error methods disclosed the fact

that ingestion of certain commercially prepared cake icings, certain fillings in candies, and certain brands of cream cheese known to contain the vegetable gums had invariably resulted in symptoms of epigastric distress, belching, flatulence, and a most disturbing severe diarrhea.

The following experiment was undertaken: The patient was given powders each containing 300 mg. of the mixed gums, namely, karaya, tragacanth, and arabic, to be taken every two hours. Six hours after the initial ingestion of the powders (after an intake of 900 mg.) there resulted a soft stool. After an intake of 1,200 mg. there resulted a definite diarrhea; there were also associated symptoms of epigastric distress, belching and flatulence. A single dose of 2,100 mg. of the powders produced, within three hours, all of the symptoms stated above.

*Ingestion Experiment: Case 9 (B. D.)** had been found markedly sensitive to karaya, tragacanth, and arabic. Ingestion of ice cream and frozen custard known to contain the vegetable gums produced symptoms of vasomotor rhinitis and bronchial asthma. Ingestion of the same foods free from the vegetable gums was normally tolerated.

The following experiment was carried out: on the morning of June 5, 1948, the patient was given powders, each containing 300 mg. of mixed gums, namely, karaya, tragacanth, and arabic, every two hours. Soon after the intake of the powders, the patient suffered marked nasal congestion, rhinorrhea, and blocked nasal passages. After the third dose of the powders she sensed definite constriction in her chest, with audible wheezy respirations. Asthmatic breathing was marked at about 3 P.M. That afternoon the patient was forced to leave school and was confined to the house the rest of the day. She continued to take the powders in the late afternoon and, after having taken 1,500 mg., the symptoms of vasomotor rhinitis and bronchial asthma became markedly and progressively intensified. She was forced to resort to ephedrine sulfate capsules for relief of the symptoms.

*Ingestion Experiment: Case 6 (A. S.)** had manifested symptoms of severe vasomotor rhinitis when applying certain brands of tooth paste as a dentifrice. The patient was found markedly sensitive by direct skin test and by passive transfer to the vegetable gums, karaya, tragacanth, and arabic.

The following experiment was carried out on this subject: At 5 P.M. of May 10th, the patient was given a total of 2,100 mg. of mixed gums in powder form, with one glass of water. At 5.20 P.M., there occurred marked clogging of the nose, continuous marked rhinorrhea and profuse discharge, and a good deal of sneezing. Examination of the nose revealed bilateral edema of the mucous membranes; the right inferior turbinate appeared greatly engorged and water-logged. There was marked obstruction on both sides of the nose due to the edema, rhinorrhea, and mucous discharge.

The control subject was G. L., 15 years of age. The skin test to karaya was 4 plus and to tragacanth and arabic, respectively, 2 plus and 1 plus. This subject gave a negative history regarding the ingestion of gum-containing foods. He was considered clinically nonsensitive to the gums.

*See Table I.

On the same day and at the same time the ingestion experiment was carried out on patient A. S., the control subject was given a similar amount of the gums (2,100 mg.) in powder form. The result was negative.

*Ingestion Experiment: Case 10 (L. D.)** had been clinically sensitive to the following gum-containing foods: salad dressings, shrimp sauce, and other sauces known to contain the vegetable gums. The following events served as proof in this subject:

The direct skin test with karaya was moderate; to tragacanth, it was marked; and to arabic, it was markedly active.

TABLE IV. INGESTION EXPERIMENTS: SUMMARY TABLE

SUBJECT	AGE AND SEX	FEEDING METHOD EMPLOYED IN REPRODUCING SYMPTOMS	OTHER METHODS OF REPRODUCING SYMPTOMS	CLINICAL MANIFESTATIONS OF ALLERGY PRODUCED	TIME INTERVAL OF ONSET; DURATION OF SYMPTOMS
R. L.	22 F.	Vegetable gums in fillings of candies on top layer; no gums on bottom layer; layers interchanged	Feeding cream cheese containing gum, controlled by other types not containing gum, and marshmallows	Epigastric distress, belching, flatulence, nausea	Onset within minutes; lasted many hours
J. G.	29 M.	May 28-29: 300 mg. mixed gums in powder form every 2 hrs. Total: 1,200 mg. June 10: 2,100 mg. mixed gums in powder form, in one dose	Gum-containing cake icings, certain fillings in candies, certain brands of cream cheese	Epigastric distress, belching, flatulence, diarrhea	Onset within 4 to 6 hrs.; lasted 12 hrs.
A. S.	13 M.	2,100 mg. mixed gums, powder form, in one dose, with one glass of water	Certain brand of tooth paste	Severe acute symptoms of vasomotor rhinitis	Onset in 20 min.; lasted 1 hr.
B. D.	16 F.	300 mg. mixed gums in powder form every 2 hrs. Total: 1,500 mg.	Gum-containing ice cream, frozen custard	Vasomotor rhinitis, bronchial asthma	Onset within 6 hrs.; lasted 24 hrs.
L. D.	55 M.	2,100 mg. mixed gums in powder form with one glass of water	Gum-containing salad dressings, shrimp sauce, etc. Intradermal tests with the gums	Severe acute symptoms of vasomotor rhinitis	Onset within 10 min.; lasted 3 hrs.

In this summary table of the ingestion experiments we note particularly the following: in the cases of R. L., A. S., and L. D., the onset of symptoms occurred within minutes and lasted many hours in the first and last instances, and one hour in the case of A. S.

In the cases of J. G. and B. D., the onset of symptoms occurred within four to six hours, and lasted twelve hours in the former and twenty-four hours in the latter.

*See Table I.

On June 10, 1948, when tested intradermally with these gums for the first time, a reaction manifested itself in the form of severe vasomotor rhinitis, characterized by marked nasal congestion, rhinorrhea, clogging of the nasal passages, and severe sneezing. This reaction lasted three hours and was only slightly ameliorated by Trimeton.

On June 22, 1948, the patient was examined and his general condition was found excellent; the condition of the nose and throat were also excellent. He was again tested intradermally with karaya, tragacanth, and arabic. Reactions to the skin tests were positive, similar to the previous results, and a similar reaction, severe vasomotor rhinitis, resulted. This also lasted three hours.

On July 25, 1948, the patient was given 2,100 mg. of the mixed gums, karaya, tragacanth, and arabic, with a glass of water. A response similar to the above two reactions resulted.

TABLE V. RELATION OF THE DOMINANT ANTIGENS AS DETERMINED BY SEROLOGIC STUDIES AND DIRECT SKIN TESTS TO THE OBSERVED CLINICAL MANIFESTATIONS IN FIVE HIGHLY SENSITIVE SUBJECTS

SUBJECT	DOMINANT ANTIGEN BY DIRECT SKIN TEST	DOMINANT ANTIGEN BY SEROLOGIC RESULTS	CLINICAL MANIFESTATIONS
R. L.	Tragacanth	Not available	Gastrointestinal allergy
J. G.	Tragacanth Karaya	Tragacanth Karaya	Gastrointestinal allergy
A. S.	Arabic	Arabic	Vasomotor rhinitis
L. D.	Arabic	Arabic	Vasomotor rhinitis
B. D.	Karaya Tragacanth	Karaya Tragacanth	Bronchial asthma and vasomotor rhinitis

In four of these subjects, serologic studies were made, in addition to direct skin tests for the determination of the dominant antigen. In the two subjects with gastrointestinal allergy, tragacanth and karaya were the dominant antigens. In the two subjects with vasomotor rhinitis as the allergic manifestation, gum arabic was the dominant antigen; while in the one subject with bronchial asthma and an associated vasomotor rhinitis, karaya and tragacanth were the dominant antigens.

DISCUSSION

The present report deals with allergic disorders caused by the ingestion of the vegetable gums, karaya, tragacanth, and arabic. These gums were contained in certain brands of foods to add bulk, thickness, and binding qualities, for the purpose of "enriching" the foods and making them "heavy."

From the figures supplied by various importing houses¹² regarding the amount, in pounds, of these three gums consumed annually in the United States, it would appear that, if distributed equally per capita (on the basis of the 1940 census figures for the total population of the United States), there is a possibility that each person could be exposed to an average of 300 mg. of the gums, karaya, tragacanth, and arabic per day. Therefore in our ingestion experiments we used 300 mg. of the mixed gums in powdered form, and results obtained showed that very small amounts of these substances were capable of producing severe allergic symptoms. This fact has been previously reported.¹⁰

It is also possible that on certain days the gum intake from foods for certain individuals might be doubled or even tripled; for it may happen that on a

particular day an individual would include in his ingestion menu a complete five-cent package of gum drops, some gum-established ice cream, a gum-emulsified salad dressing, a gum-containing piping or icing, a gum-rich pie filler, and similar items for which the gums are used. This would naturally result in increased allergic symptoms and would account for gradations of symptomatology from the use of the gums in certain allergic patients.

Thus our patient J. G., who reacted with gastrointestinal symptoms from the gum-containing foods, would on some occasions have suffered only belching, epigastric distress and flatulence, and perhaps only a soft stool, while on other occasions a severe diarrhea would have been an additional symptom.

In a general way the major clinical manifestations of allergy resulting from the ingestion of the vegetable gums encountered in our ten subjects were bronchial asthma, urticaria, angioedema, vasomotor rhinitis, and gastrointestinal symptoms. There was no instance of migraine, acute or chronic eczema, or other manifestations of allergy traceable to the ingestion of the vegetable gums.

The four positive sera made available in the present series lent conclusive proof of the role of the gums as sensitizing allergens in predisposed persons, and served as a means in determining the dominant antigens in these subjects.

The vegetable gums employed otherwise than in foods were the subject of a previous study,¹¹ indicating their sensitizing qualities by inhalation and external contact. It should be stressed that a considerable amount of gum arabic finds its way into adhesives, and a considerable amount of gum tragacanth is used in cosmetic and pharmaceutical products; also that large quantities of karaya are contained in hair dressing, wave set, and other lotions employed for external application. In view of the wide usage of these gums, it is necessary to include them constantly in our testing tray.

Gums chemically similar to these principal commodities are frequently employed in their stead, such as cherry tree gum as a substitute for arabic, locust as a substitute for karaya, and algin and other gums as partial substitutes for tragacanth. Similarly, to counteract untoward reactions frequently occurring from laxatives, colloid laxatives are substituted at present. Synthetic mucilages such as polyvinyl alcohol and methyl cellulose are now employed to provide hydrophilic colloids which do not produce the usual side reactions of the vegetable gums. These hydrophilic colloids are not absorbed by the intestinal mucosa, are not degraded by intestinal enzymes, and are not antigenic.¹²

The following data regarding the use of the gums in the foods consumed by our patients has been supplied to the author and, by permission, are herewith listed:¹⁴

Gravies.—A frequent practice is that of adding gum tragacanth to impart thickening ordinarily achieved with flour and starches.

White Sauce.—As in the case of gravies, gum thickeners are used, principally tragacanth.

Prepared Mustard.—Some brands are thickened with karaya or locust to prevent separation.

Processed Cheddar Cheese.—Most processed cheeses contain gums, particularly karaya, locust, and, very rarely, tragacanth.

Processed Swiss Cheese.—See *Processed Cheddar Cheese*.

Prepared Potato Salad.—The dressing is thickened with karaya.

Cake Icing Mixtures.—These contain gum arabic and, less often, either karaya or locust.

Commercial Whipped Cream.—Frequently stabilized by a mixture of sugar, cornstarch, gelatin, agar-agar, salt, and vanilla. During the war, difficulty in procurement of agar led to its replacement in many instances by either karaya, locust, tragacanth, or, most generally, sodium alginate. This practice still persists.

Ice Cream Mixtures.—These contain karaya and/or locust. Sodium alginate is used in some, but karaya is more prevalent.

Fillings in Candies.—Soft centered candies usually contain considerable gum arabic. In some instances karaya is used.

Cream Cheese.—Packaged cream cheese is labeled with an indication that it carries a "vegetable stabilizer," most always karaya.

Tooth Paste.—Some tooth paste is free from gums, but some carry uronic acid gum, generally karaya and occasionally locust as a substitute for karaya.

Certain Brands of Chewing Gum.—The scarcity of chicle during the war resulted in the development of numerous gum substitutes, and in the change of several chewing gum formulas which included then (as many still include), gums such as tragacanth and, more rarely, karaya.

Charlotte Russe.—The whipped cream filling is stiffened with a gum, which replaces the agar-agar formerly used. Karaya is the commonest choice.

Shrimp Sauce.—See *White Sauce*.

Salad Dressing.—With the exception of mayonnaise, which would be considered adulterated by the Government if it contained gums, other forms of salad dressing substitutes for mayonnaise contain thickening agents, principally karaya, locust and, less frequently, tragacanth.

Marshmallows.—Ordinarily marshmallows are gelatine confections and are devoid of added gums. Occasionally, however, gum acacia is added. When marshmallows are prepared from *Althea officinalis*, it is a trade secret to employ gum acacia to insure the rigidity of the confection.

Certain Brands of Wheat Cakes.—Few prepared griddle cake flours disclose the presence of gum in the list of ingredients. Despite this, a number of prepared flours contain small amounts of the order of one-half per cent of karaya.

SUMMARY

1. The vegetable gums, tragacanth, karaya, and arabic, may cause allergic disorders by ingestion in sensitive subjects.
2. Ingestion experiments with these gums carried out in five sensitive subjects established the etiological relationship of these allergens to the allergic manifestations produced.
3. Passive transfer tests in serial dilutions as well as neutralization studies confirmed the allergenic nature of these gums in the sensitive subjects studied.
4. Widespread employment of these gums in food and other industries is a hazard for the sensitive individual from ingestion, inhalation, and surface contact of these gums.
5. Substitution of other gums for tragacanth, karaya, and arabic, and of synthetic mucilages and celluloses will help in offsetting the danger of sensitization encountered from their use.

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DISCUSSION

STEARNS S. BULLEN, SR., Rochester, N. Y.—Allergic reactions following exposure to vegetable gums are not very frequent, but when they do occur they are likely to produce severe and explosive symptoms.

It is known that the vegetable gums produce symptoms in susceptible people by contact and by inhalation. Dr. Gelfand has given further evidence that the ingestion of these gums may result in allergic reactions.

His case reports illustrate very well the fact that an ingested substance may produce reactions not only in the gastrointestinal tract but also on the skin and the mucous membrane of the respiratory tract and further that reactions may occur in the respiratory tract without any symptoms referable to the gastrointestinal tract. Also in one of his cases, although there was a four plus reaction on skin testing, no symptoms occurred on exposure to the gum. While all these phenomena are well known, it is seldom that they are so well illustrated in a small group of cases.

The constitutional reaction occurring on skin testing in one case which was intentionally reproduced should serve as a warning that skin testing with these vegetable gums is not without danger and should be done with the same care as with extracts of fish, cottonseed, and so forth.

Dr. Gelfand's list of substances in which these vegetable gums are used on a commercial scale will be of great aid to the clinician.

GROWTH OF RABBITS ON PURIFIED DIETS¹

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The growth of rabbits fed casein-sucrose purified diets is usually less than optimum, and certain natural feedstuffs must be added for good gains. Hogan and Hamilton ('42) reported that rabbits grew normally on casein diets supplemented with yeast or liver extract. Kunkel, Simpson, Pearson, Olcese, and Schweigert ('48) also noted the beneficial effect of liver extract for rabbit growth and maintenance of the fur. Wooley ('54) reported that leafy material, such as kale, added to the casein diet greatly improved growth. Good growth was also obtained by readjusting the potassium level of the diet and increasing the casein level to 30%. The beneficial effect of soybean meal will be reported in the present paper. The rabbit has a high potassium requirement (Hove and Herndon, '55) and perhaps a high sodium and calcium requirement (Wooley and Mickelsen, '54); since little is known about the quantitative requirements of other nutrients it is hard to assess the reasons for the growth stimulation brought about by certain feed ingredients.

EXPERIMENTAL

The compositions of rabbit diets R43 and R14E are given in table 1. Variations in the level of protein source or of

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supplements were compensated by adjustments in the carbohydrate level. Commercial, solvent-process soybean meal that was further subjected to a one- or 2-day continuous methanol extraction was used throughout ($N \times 6.25 = 51.3\%$ protein). The casein was also methanol-extracted.

Rabbits of the New Zealand-white or the California-white strains were placed on the experimental diets at 4 weeks of

TABLE 1
Composition of diets

	K-43	K-44
Casein ¹	20	0
Soybean meal ¹	0	40
Sucrose	51	32
Celulose ²	10	10
Salt mixture no. 5 ³	5	5
Potassium bicarbonate	1	0
Lard	6	6
Cod liver oil	2	2
Vitamin premix ⁴	5	5

¹ Continuous one- or two-day methanol extracted.

² "Non-nutritive fiber" of General Biochemicals, Inc.

³ W. D. Salmon, *J. Nutrition*, 22: 155 (1917).

⁴ Contributed per gram diet: thiamine, riboflavin, pyridoxine, 3 μ g each; calcium pantothenate, 17 μ g; choline chloride, 2,000 μ g; inositol, 200 μ g; niacin, 30 μ g; methoxy-L-naphthalenol, 0.3 μ g. (Vitamin B₁₂, 20 μ g/kg and folic acid, 2 mg/kg were added to some of the diets: expts. II, III and IV.) *d,l*- α -Tocopherol acetate was given as 10 ng per week, orally.

age and at body weights usually of 400 to 500 gm. The animals were housed individually on half-inch mesh screens in an air-conditioned room. Water and feed were always available to the animals.

RESULTS

The normal rate of weight gain of young rabbits fed a ground, commercial rabbit feed⁴ with 18% protein was about 35 gm/day (table 2). The simplified diet containing casein as the protein source, as used in the series I experiments, did

⁴ See Rabbit Pellets.

not produce growth equivalent to that of the commercial feed even when the casein level was raised to 50%. The data in table 2 indicate that this high level of casein was superior to lower levels. However, the rate of gain was not maintained past 6 to 7 weeks on this diet. About one-third of the animals fed the casein diets showed marked loss of fur, regardless of

TABLE 2
Rabbit growth on purified diets

(Initial body weights 400 to 600 gm; time on diets usually 32 days, except for amino acid studies which were run 14 days)

EXPERIMENTAL SERIES NO.	LEVELS OF PROTEIN SOURCE AND ADDITIONS	NO. OF RABBITS	RATE OF GAIN
I	%		gm/day \pm S.E.
	Casein, 10	4	4.8 \pm 0.7
	Casein, 20	10	10.4 \pm 2.2
	Casein, 33	4	12.4 \pm 1.9
	Casein, 50	4	23.4 \pm 2.2
	Soybean meal, 40	5	27.2 \pm 2.7
	Casein, 20 + soybean meal, 15	5	29.6 \pm 2.0
II			
	Casein, 20	12	14.2 \pm 2.1
	Casein, 33	10	15.7 \pm 2.5
	Soybean meal, 40	8	32.2 \pm 2.3
	Casein, 20 + soybean meal, 25	6	35.3 \pm 2.2
	Casein, 20 + water-insol. res. soybean meal, 25	2	31.5
	Casein, 20 + tryptic-digest of soybean meal, 16	5	19.1 \pm 1.0
	Casein, 20 + ash of soybean meal, 4	4	15.6 \pm 1.9
	Casein, 20 + dried brewers' yeast, 25	2	36.2
	Casein, 20 + arginine, 0.8; glycine, 0.8; methionine, 0.5; tryptophan, 0.1	4	23.6 \pm 1.7
	Casein, 20 + arginine, methionine, tryptophan	2	18.0
	Casein, 20 + arginine, tryptophan	2	16.2
	Casein, 20 + glycine	2	12.0
III			
	Casein, 18	4	4.2 \pm 1.2
	Enzymatic digest of casein, 18	4	15.3 \pm 0.8
	Digest of casein, 18 + arginine, glycine, methionine	4	20.1 \pm 1.9
	Soybean meal, 36	4	30.5 \pm 3.5
IV			
	Casein-casein digest 1:1, 18	4	16.9 \pm 2.4
	Casein-casein digest, 18 + arginine, glycine, methionine, tryptophan	7	20.8 \pm 1.1
	Commercial rabbit feed (ground)	4	34.3 \pm 1.5

casein level. Simplified diets containing soybean meal as the sole protein source, or added to the 20% casein diet, permitted growth in rabbits at a rate only slightly less than that of the commercial feed.

The growth-promoting properties of soybean meal could not be removed by water extraction (experiment series II, table 2). The water-soluble extract of the soy-bean meal was dried and added to the casein diet at a 3% level, but it did not improve growth (these data not shown). A water suspension of finely ground soybean meal was digested for 6 days with 1% trypsin (1-309). The filtrate was dried and fed at a 16% level; this gave a significant but not maximum growth stimulus. Dried brewers' yeast was as effective as soybean meal. The ash of soybean meal did not stimulate growth.

The additions of the amino acids arginine, glycine, methionine, and tryptophan to the 20% casein diet resulted in a significant growth increase. However, amino acid supplementation produced erratic results, and in a rather large number of other trials no benefit at all was apparent. This contrasts with the consistent and dramatic response to soybean meal.

In some experiments (as in III, table 2) an 18% casein level, with only 3% of cellulose, was used. The substitution of an enzymatic digest of casein² for the extracted casein improved growth significantly. The addition of the amino acids arginine, glycine, and methionine to this diet resulted in a further improvement of growth, but the growth rate did not equal that produced by the soybean meal diet. Reported growth factors, such as thioctic acid (10 mg/kg diet) and orotic acid (100 mg/kg diet), were included in the vitamin mixture used for experiment III; no benefit from these factors could be discerned.

The dried ceca (with contents) of rabbits fed the 20% casein diet were significantly heavier than those of the animals fed the soybean meal diet. Expressed as percentage of body weight, these values for 6 animals/group were 2.71 ± 0.15 .

²Additional Biochemicals, Inc., Chagrin Falls, Ohio.

and 1.73 ± 0.30 , respectively. This may indicate a type of intestinal stasis with slower passage of ingesta through the tract.

The effect of the type of carbohydrate in the diet on the rate of body weight gain of rabbits is shown in table 3. All diets contained at least 5% sucrose, since this was the carrier for the vitamins. When corn starch was substituted for sucrose a slight but statistically insignificant improvement in growth resulted, regardless of whether the protein source was

TABLE 3
Effect of type of carbohydrate on rabbit growth
(4 rabbits per group with average initial weights of 350 to 410 gm; 40 days on diets)

PROTEIN SOURCE	CARBOHYDRATE ADDED TO DIET	RATE OF WEIGHT GAIN
20% Casein	Sucrose 54, cellulose 10	11.5 ± 0.93
	Cornstarch 54, cellulose 10	15.1 ± 1.88
	Cornstarch 64	13.7 ± 0.90
	Sucrose 34, cellulose 10, gum arabic 20	16.7 ± 1.28
40% Soybean meal	Sucrose 34, cellulose 10, agar 20	21.8 ± 1.55
	Sucrose 34, cellulose 10, triacetin 20	5.9
	Sucrose 32, cellulose 10	21.7 ± 1.88
	Cornstarch 32, cellulose 10	20.4 ± 1.44
	Cornstarch 42	25.2 ± 2.01

¹ Twenty-eight-day period.

casein or soybean meal. Omission of cellulose from either diet did not interfere with the growth rate. The addition of either gum arabic or agar, at a 20% level, resulted in significant improvement in growth rate in rabbits fed the casein diet. This is similar to the observation of Booth, Elvehjem, and Hart (149) with guinea pigs. Triacetin-containing diets depressed rabbit growth.

The total carbohydrate of soybean is composed, according to Markley and Goss (144), of about 4 to 6% each of cel- sucrose, stachyose, galactan and arabin; only slight traces of starch or hemicelluloses are present. The inclusion of 25%

soybean meal in a 20% casein diet more than doubled the growth rate. This amount of soybean meal contributed about 10% of carbohydrate, 13% of protein, and 2% of ash. Since most of the carbohydrate consisted of substances known not to influence rabbit growth, such as sucrose, cellulose and starch, it is doubtful that the carbohydrate is a significant factor in the stimulation of growth. However, the galactans and arabans of soybean may have an effect on rabbit growth similar to that of gum arabic or agar.

DISCUSSION

The protein of soybean meal contains considerably more arginine and glycine than does casein. Supplements of these amino acids along with some methionine and tryptophan to the casein diet produced results that were erratic but positive enough to indicate that a partial deficiency of these amino acids contributed to the relatively poor growth on casein diets. Involvement of amino acids was further indicated by the better growth obtained when an enzymatic digest of casein replaced casein.

However, the erratic response would indicate that some nutrients other than the amino acids are limiting in the casein diet and are contributed by the soybean meal or yeast supplement. Almost nothing is known of the quantitative requirements of the rabbit for minerals or vitamins except for potassium (Hove and Herndon, '55); choline (Hove and others '55; pyridoxine (Hove and Herndon, '57) and niacin (Wooley, '47). Perhaps certain minerals or vitamins are supplied to the diet in insufficient or imbalanced amounts by the mineral and vitamin mixtures which, after all, are patterned upon the requirements for the growth of rats.

SUMMARY

For growth of rabbits fed a simple purified diet, defatted soybean meal was superior to casein as a protein source. With 20% casein the rate of growth was about 15 gm/day. Soybean as the sole source of protein at an equivalent level

permitted growth of about 30 gm/day. This compared favorably with growth of 35 gm/day for rabbits on a commercial feed. A supplement of arginine, glycine and tryptophan improved growth of rabbits fed the casein diet, and accounted for a part of the soybean meal effect. Other deficiencies in casein, as yet unidentified, were corrected by soybean meal or by dried brewers' yeast.

The type of carbohydrate in the diet had a minor effect on rabbit growth. Sucrose, starch, gum arabic, agar, cellulose and triacetin were compared. Gum arabic and agar influenced the growth rates most favorably.

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STABILITY OF VITAMIN A IN THE SHARK-LIVER OIL EMULSION

Shark-liver oil emulsion containing gum acacia showed a rapid loss of vitamin A content on storage but the activity was preserved either by protecting the emulsion from light or by heating the acacia at 100° for an hour or by adding one per cent of α -tocopherol to the emulsion.

Shark-liver oil emulsion is frequently prepared with acacia as an emulsifying agent. It has been reported¹ that acacia contains an oxidase-type enzyme which renders it unsuitable for use in pharmaceutical preparations containing easily oxidizable active constituents. Vitamin A is sensitive to light and to oxidizing agents². Kedvessy³ observed that vitamin A content of cod-liver oil emulsions made with acacia decreased to 54 per cent in three weeks. Griffiths *et al*⁴, on the other hand, demonstrated that cod-liver oil emulsions can be kept for at least a few months without any serious loss of vitamin A, if stored in a well-stoppered, amber glass bottle and kept in the dark.

Tocopherol is known⁵ to be an effective antioxidant for vitamin A in oily preparations but the studies on its effect in aqueous dispersions have yielded conflicting results. Patel and coworkers⁶ find that tocopherol stabilized vitamin A in oily media but had no significant effect in aqueous dispersions. Kern and Antoshkiw⁷ showed that tocopherol preserved the activity of vitamin A in aqueous dispersions at pH 4 to 5. The latter observations were, confirmed by Fatterpekar and Ramasarma⁸ who reported that 0.3 per cent of DL- α -tocopherol acetate protected vitamin A in aqueous dispersions over the pH range 2.5-6.5 and that 1 per cent tocopherol exerted maximum antioxidant effect. It was, therefore, considered of interest to undertake a comparative study of the effects of acacia, light and added antioxidant like tocopherol on the stability of vitamin A in shark-liver oil emulsion.

Shark-liver oil (Sharkovit) marketed by Fisheries Technological Laboratory, Bombay, and DL- α -tocopherol acetate, B.P.C. made by F. Hoffmann-la Roche and Co., Basle, were used.

An emulsion of shark-liver oil was prepared with the help of an electric stirrer. The shark-liver oil (100 ml.) and chloroform 0.4 ml. were emulsified with a mucilage of acacia (25 g.) and tragacanth

(1.4 g.) in distilled water (50 ml.). Sodium benzoate (1.0 g.) dissolved in water (5 ml.) was then incorporated and the emulsion was diluted with distilled water to 200 ml. The product was further homogenized by passing thrice through the Q. P. Hand Homogeniser (Ormerod Engineers, Rochdale, England).

Two 100 ml. aliquots of this emulsion were packed in a colourless bottle (A) and in an amber-coloured bottle (B). Another sample of the emulsion prepared as above with the addition of 1 per cent tocopherol (C) was packed in a colourless bottle. Another sample of the emulsion (D) was prepared in the same way but using powdered gum acacia, previously heated at 100° for an hour, and stored in a colourless bottle. A sample of shark-liver oil itself (E) was set aside in its original container (amber-coloured bottle) for comparison. All these samples were stored at room temperature (30-35°). The emulsions were found to contain 81.2 million globules per cu. mm. and the root mean cube diameter of the globules⁹ was 2.86 μ .

For the determination of vitamin A, the shark-liver oil was extracted from the emulsion with ether (peroxide-free¹⁰) by the method of Griffiths *et al*⁴. The potency of vitamin A in the unsaponifiable fraction of the shark-liver oil was measured spectrophotometrically by the B. P. method¹¹ on a Hilger and Watt's Uvispek photoelectric spectrophotometer. As reported by Wai *et al*¹², the vitamin A in shark-liver oil emulsion did not show its absorption maximum at 326.5 $m\mu$. However, estimation by taking readings at this wavelength is considered to be satisfactory for the study of percentage loss on storage.

The vitamin content in each sample was determined after storage for 2, 4 and 8 weeks. The percentages of vitamin A remaining after 8 weeks' storage in samples A, B, C, D and E were 60.0, 98.4, 98.6, 98.0 and 99.3 respectively. These results show that the vitamin A content of shark-

liver oil emulsion decreased appreciably only in sample A (containing gum acacia and stored in a colourless bottle) while in all other samples there was negligible loss of vitamin A.

These results emphasize the advantage of pre-heating powdered acacia at 100° for an hour or storing the emulsion in an amber-coloured container or adding α -tocopherol as an antioxidant to the emulsion.

Our thanks are due to Dr. K. N. Gaiind, Head of the Department of Pharmacy, Panjab University, for his keen interest during the course of this investigation.

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Non-Availability of Gum Acacia as a Glycogenic Foodstuff
in the Rat.

J. VICTOR MONKE. (Introduced by John C. Krantz, Jr.)

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No biochemical analyses have been made to test whether the gum acacia molecule passes entirely undigested through the gastrointestinal tract. Observations on poorly controlled feeding experiments, cited in the older literature,¹ are reported to have shown that gum acacia, without other dietary supplement, is a deficient foodstuff. Since gum acacia is a rather important physiological tool,¹ any information concerning its chemistry or physiological properties is of some interest.

The question arose whether all of the sugar components of the molecule² are tightly bound into a "main" chain molecule, or whether some of them are possibly attached as "side" chains to the main molecule. In the latter case, they might be vulnerable to the digestive enzymes, and thus become materials available for glycogenesis.

¹ Amberson, W. R., *Biol. Rev.*, 1937, 12, 48.

² Butler, C. L., and Cretcher, L. H., *J. Am. Chem. Soc.*, 1929, 51, 1519.

Two groups of 15 young male rats (avg. wt. 140 g) were removed from a normal diet of dog mash, placed in individual cages, and fasted for 48 hours. Five individuals in each group were given 10 g of cacao butter, the remaining ten, 10 g of a mixture containing 34% of white powdered gum acacia (Arthur H. Thomas) and 66% of cacao butter. Tests for free reducing substances in the gum acacia were negative. At the end of 72 hours each rat was anesthetized with 0.5 cc of 10% sodium amytal administered intraperitoneally. The liver was immediately extirpated and placed in a tared 50 cc centrifuge tube containing 15 cc of 30% potassium hydroxide. Glycogen was obtained by means of Good's modification³ of Pfluger's method. Glucose was determined upon an aliquot of the hydrolyzed glycogen according to the method of Shaffer and Hartman.⁴

The livers of the 2 control groups contained an average of 0.15% and 0.13% glycogen by weight, respectively, while those of the acacia-fed groups contained an average of 0.24% and 0.17% glycogen by weight, respectively. The variation in the percent of glycogen by weight between the livers of the individual rats in the control groups ranged from 0.03% to 0.29%, with a mean in each group of 0.12% and 0.17%, respectively. Variations in percent glycogen by weight in the livers of the acacia-fed groups ranged from 0.04% to 0.31% in the various individuals. These had a mean value for each group of 0.27% and 0.18%, respectively.

In previous papers reported by Krantz, *et al.*,^{5, 6} it has been established that the assay of glycogenic activity on the part of a compound administered as it was in these experiments does not become significant unless the percentage increase has an average value of the order of 300% or more.

It is concluded that the difference in liver glycogen between the control and the acacia-fed rats is insignificant. No part of the gum acacia molecule is subject to disintegration by the enzymes of the digestive tract of the rat. Orally administered gum acacia is excreted unchanged in the feces.

³ Good, C. A., Kraemer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, 100, 485.

⁴ Shaffer, P. A., and Hartmann, A. F., *J. Biol. Chem.*, 1920, 45, 349.

⁵ Carr, C. J., Musser, R., Schmidt, J., and Krantz, J. C., Jr., *J. Biol. Chem.*, 1933, 102, 721.

⁶ Krantz, J. C., Jr., Evans, W. C., and Carr, C. J., *Quart. J. Pharm. and Pharmacol.*, 1935, 8, 213.

Nutrition

NUTRITIONAL STUDIES OF COMPLEX CARBOHYDRATES. G. M. Shue*,
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The caloric value of agar-agar, gum arabic and cellulose was measured according to the technique of Rice et al (J. Nutr. 61, 253 (1957)). Weanling rats were trained to consume the daily portion of experimental diet within one to two hours. The supplements studied were added to 5 grams of basal ration [Sucrose 42.7%, casein 40.0, cystine 0.3, USP salt mix 6.0, vitamin-Cellulofour mix 6.0, cottonseed oil 2.6, vitamin ADEX (cottonseed oil solution) 2.4%] in amounts of 0.25, 0.5, 1, 2 and 4 grams. Feces collections allowed concurrent determination of apparent dry matter digestibility. The feeding of agar-agar under these conditions produced a growth depression directly related to the level of agar fed. Fecal nitrogen was doubled at all levels of supplementation. Digestibility data suggests that more than 15% of agar was absorbed and that up to 80% of gum arabic was absorbed. The growth data for gum arabic shows a negative regression of weight gain on dose. At a dietary level of 16%, the weight gain observed is approximately 75% that observed with sucrose. The growth data does not indicate any caloric contribution from cellulose.

Source: Z. Lebensm.-Untersuch. & Forsch. 104, 187-192 (1956)

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IDENTIFICATION BY PAPER CHROMATOGRAPHY
OF SWELLING AGENTS IN FOODSTUFFS

Testing for swelling agents or thickeners in pure form presents no difficulties. A compilation by Letzig (1) lists a number of precipitating reactions which permit distinguishing between the several thickeners in simple manner. However, these compounds seldom exist in such pure form that the use of such precipitating reactions, presented in synoptic form, permits non-ambiguous conclusions. Even the occurrence of two different swelling agents simultaneously can make a clear identification difficult; when there are more than two, this is true even more so. However, not only a mixture of these substances complicates the tests but also the presence of other compounds among which the proteins are predominant. Letzig's reagents are mainly protein precipitants so that precipitation will always occur in the presence of proteins. It is easy, of course, to determine the presence of proteins but this does not yet allow us to decide whether, in the absence of proteins, precipitation will have been due to thickeners. Prior to testing, proteins must therefore be removed and Wyler (2) has already described a possibility for this, specifically for meat products. This suggested removal of proteins with the aid of Carrez precipitation is so thorough, however, that not only are many swelling agents precipitated for which we must thus test prior to precipitation but the possibility of identifying those still remaining in solution is also restricted to a very few. A method of complete removal of proteins without interfering with subsequent testing and identification of swelling agents is not yet known so that the Letzig reagents in practice unfortunately have only a limited importance. As seductive as such tests are in their simplicity, they alone very rarely furnish non-ambiguous

indications of the kind of the swelling agents present. There is a possibility of precipitating the proteins with alcohol which can subsequently be easily evaporated (e.g. in vacuum) but this has the disadvantage that the proteins are not always completely eliminated and that some thickeners, specifically carob-seed flour, are also precipitated.

These disadvantages raise the question of different testing methods and Letzig already indicates in the report cited that the application of paper chromatography promises success. Since most thickeners are constituted by carbohydrates, no difficulties exist from the point of view of paper chromatography; it is merely necessary to convert the test substance into a form suitable for paper-chromatographic testing. It may happen, however, that with several swelling agents present, a non-ambiguous conclusion on the kind of the individual substances is not possible since a number of thickeners have a similar composition and/or furnish similar cleavage products after hydrolysis. In that case, however, a combination with the precipitating reagents of Letzig would permit us to continue further since the decision will lie between the few still possible thickeners. Whereas the proteins do not interfere with such paper-chromatographic tests, we must first test for the presence of carbohydrates not originating from swelling agents. Admixtures of simple sugars easily determined with the aid of paper chromatography, can generally be removed by extracation with 50-% alcohol which does not affect the swelling agents. It is sufficient to let the substance stand with the alcohol for several hours under occasional shaking. The extract and/or the filtrate can serve to test for sugar (3) whereas the thickeners can be determined from the residue.

Hydrolysis of Swelling Agents

Since thickeners do not directly respond to paper chromatography, it is necessary to convert them to a form which is accessible to this method of testing. Among the possibilities of hydrolytic cleavage, that using enzymes is excluded and,

since carbohydrates are involved, acid hydrolysis is indicated primarily in order to prevent decomposition of cleavage products. The following conditions have been shown to be suitable and have been steadily employed for some time:

10 g of pure swelling agent and/or test substance are diluted with 50 ml of water and 50 ml of a 10-% sulphuric acid solution. If the mixture is too thick so that the retort may burst under direct heating, it should be placed in a hot water bath for a short time. When fluid, the solution is boiled in a reflux condenser where duration is a function of the kind of the probable thickeners since each substance is hydrolyzed at different rates of speed. If no indication of the type of swelling agent exists, the solution is kept at boiling temperature for three hours. It can then be directly chromatographed. However, it is preferable to concentrate if there is only a small amount of test substance; sulphuric acid also interferes somewhat, specifically with uronic acids, so that it is preferable to remove the former. For this purpose, 30 g barium hydroxide (with 8 mol water) are dissolved in 100 ml of water under heating and dripped, under constant shaking, through a filter into the hot hydrolytic solution until the latter reaches a p_H of 7. With these generally known conditions for removal of the sulphuric acid, it is easy to adjust the p_H -value as above by spotting Merck indicator paper. The precipitated barium sulphate can be rapidly filtered out, after settling, through a standard filter. Since only small amounts are required, it is sufficient if a part of the filtrate is processed further. This part is concentrated under vacuum to 50% and thus reaches again the starting concentration. If only small amounts of swelling agent are available, these can be further concentrated as desired. This solution is now suitable for paper chromatography. It is not at all necessary to keep within the quantitative relations indicated above and they can all be reduced correspondingly which is of value when only small amounts of test substance are available.

Paper-Chromatographic Conditions

Paper chromatography always takes place under the conditions already described earlier (3 & 4). The fluxing agent is n-butanol/pyridine/water (3:2:1.5) and staining is done with phthalic acid/aniline and/or naphthoresorcinol/trichloroacetic acid. The last-named staining for non-reducing sugars plays a subordinate role in this connection. The chromatograms thus obtained with the ascending method are shown in the figure at the end of the article. The parallel obtained test solutions permit non-ambiguous identification so that even closely adjacent spots cannot be confused with each other which may easily occur when working exclusively with R_f -values.

Evaluation

Fig. 1 shows two chromatograms of some frequently occurring swelling agents. It will be clearly seen that, after hydrolysis of carob-seed flour, only the spots of galactose and mannose appear. Although mannose is nearly equal in R_f -value to mannose, it can be distinguished from the reddish arabinose by its brown color tone but these colors cannot be recognized in a black-and-white photo. With agar-agar, only galactose is clearly seen whereas xylose is manifested only as a weak spot. Gum arabic shows galactose in addition to arabinose and rhamnose. Although rhamnose is recognized only vaguely, it is characteristic since it is only infrequently found in thickeners. In the sample examined by us, there occurs further a spot immediately below the starting point which we were never able to identify. Pectin shows mainly the spot of uronic acid which can be recognized from the typical light-brown color after staining with phthalate/aniline as well as by staining with naphthoresorcinol/trichloroacetate when it turns slightly blue after some time (cf. 3 for details). We know that this involves galacturonic acid which paper chromatography cannot demonstrate since sufficient separation from glucuronic acid does not take place. The R_f -values of the two uronic acids do differ slightly and we might therefore assume that differentiation would be possible after sufficient time. Here we must take into account that the R_f -values of the two

acids are to a considerable extent a function of the concentration of these acids and of the kind of an eventually existing cation. Something similar has already been shown for some other organic acids (5). The variations so caused are greater, however, than the difference between the two R_f -values. In addition to the spot of galacturonic acid, a weak spot of glucose also occurs with pectin. It must be remembered further that a duration of hydrolysis of three hours must be strictly kept, in contrast to most other swelling agents. Whereas a duration of hydrolysis of one to two hours generally produces complete cleavage in the latter, galacturonic acid in pectin shows only weakly after one hour but increases with continued hydrolysis. Tragacanth gum shows the spots of galactose, arabinose and xylose. Comparison with the test solutions which were run parallel on the left chromatogram, makes possible rapid identification of the individual spots. The first test solution on the left shows the upper spot as galactose and the lower spot as mannose; the second test solution indicates the upper spot as arabinose and the lower as rhamnose. We already mentioned that arabinose and mannose cannot be differentiated in the figure since it is not possible to see the differences in color. The chromatogram makes it impossible to mix up the red color of arabinose with the brown color of mannose. The last test solution shows galacturonic acid at the top and, below this, xylose.

The chromatogram on the right of the figure shows "tylose" (methyl cellulose) on the left side which shows as expected the spot of glucose and below this non-hydrolyzed methylated glucoses. The occurrence of these methylated glucoses was in several preparations and appears to be characteristic for most of the tylose preparations. It is possible, however, that this is a function of manufacture and that these spots may therefore be absent some times. The adjacent cellulose glycolate also shows the spot of glucose and, in the upper part of the chromatogram, two and occasionally three unidentified spots which always have a characteristic semilunar form. Alginate exhibits only the spot of a uronic acid. The adjacent test solutions show in the

first case from top to bottom the spots of lactose, maltose and glucose whereas glucuronic acid is shown on the left.

When several thickeners are present, identification on the basis of the chromatogram can become difficult. It then seems preferable to make identification less through the spots that do show but initially through those absent. The absence of uronic acid permits a definite conclusion that neither pectin nor alginate exist. However, we believe sufficient concentration of the hydrolyzed solution must be made in order to avoid that a uronic acid does not show by reason of excessive dilution. The absence of galactose excludes the presence of carob-seed flour, agar-agar, gum arabic and tragacanth. Absence of glucose means that neither tylose nor cellulose glycolate, generally also no pectin and obviously no swelling starch can be present. Lack of mannose would exclude carob-seed flour and that of arabinose would eliminate gum arabic and tragacanth. Here we must take into account, however, that the presence of one of the two last-named spots does not permit a conclusion on the absence of the other since a possible superposition may exist which does not allow this.

With this method of selection, most of the known swelling agents can easily be excluded so that we do obtain in general a non-ambiguous picture of the composition of the mixture of swelling agents. Where in complicated cases several interpretations are possible, we can turn to the precipitating reactions Letzig mentioned above for clarification of any remaining doubts, in spite of the interferences indicated. When direct interpretation is not possible, the method of selection has proved itself also for the Letzig reactions. In most cases, however, paper chromatography should be sufficient or at least give decisive indications. As in most investigative methods, a certain intuitive judgment will develop with practice.

As described, such testing for swelling agents is obviously restricted only to those whose composition includes carbohydrates. Although these represent the greater number, this does not allow us to neglect others, e.g., gelatine. Testing for such substances

by specific paper chromatography would evidently have to be carried out from other viewpoints.

Conclusion

1. The precipitating reactions in testing for swelling agents are excellent for pure compounds but rarely furnish non-ambiguous findings for mixtures and in the presence of other substances, e.g. proteins.

2. Since most of the swelling agents are constituted by carbohydrates, paper chromatography of the sugars and/or sucroid compounds offers the possibility of identifying swelling agents by hydrolytic cleavage.

3. Hydrolysis is made with sulphuric acid and boiling at an average duration of 3 hours. After removal of sulphuric acid by barium hydroxide and concentration of the clear filtrate under vacuum, the test is made with a method described in an earlier communication.

4. Evaluation is best made by a method of selection in which all swelling agents are excluded which cannot exist due to the absence of a spot. However, it is necessary to take into account the ratios of concentration.

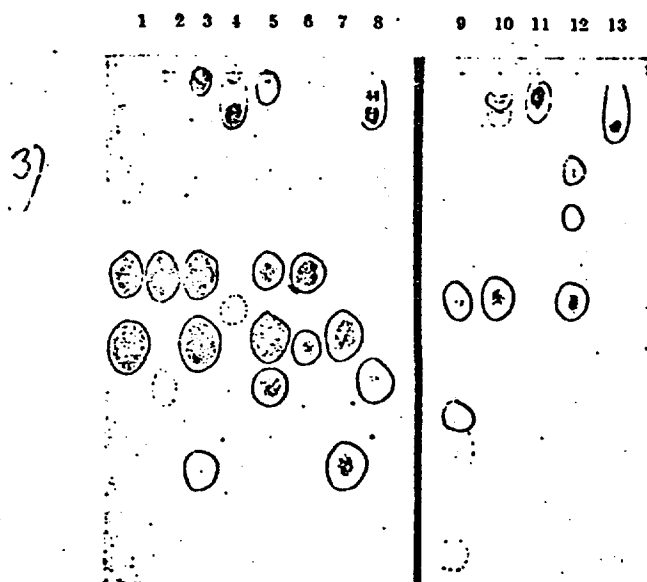


Abb. 1. Mit Schwefelsäure hydrolysierte Quellstoffe und entsprechende Testlösungen. Fließmittel: n-Butanol/Pyridin/Wasser (3:2:1.5), Färbung: Phthalsäure/Anilin. Linkes Chromatogramm: (von links) 1 Johannisbrotkernmehl, 2 Agar-Agar, 3 Gummi-arabicum, 4 Pektin, 5 Tragant. Testlösungen: 6 Galaktose + Mannose, 7 Arabinose + Rhamnose, 8 Galakturonsäure + Xylose. Rechtes Chromatogramm: (von links) 9 Tylose, 10 Celluloseglykolat, 11 Alginat. Testlösungen: 12 Lactose + Maltose + Glucose, 13 Glucuronsäure

Fig. 1 - Chromatogram of swelling agents hydrolyzed with sulphuric acid and the resulting test solutions. Fluxing agent: n-butanol/pyridine/water (3:2:1.5). Staining: Phthaleic acid/aniline. Left side: 1= carob-seed flour; 2 = agar-agar; 3 = gum arabic;

4 = pectin; 5 = tragacanth; test solutions: 6 = galactose + mannose; 7 = arabinose + rhamnose; 8 = galacturonic acid + xylose. Right side: 9 = tylose; 10 = cellulose glycolate; 11 = alginate; test solutions: 12 = lactose + maltose + glucose; 13 = glucuronic acid.

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COMMENTS ON THE MECHANISM OF THE FETOTOXIC EFFECT OF TRAGACANTH

(Über den Mechanismus der fetalschädigenden Wirkung von Traganth)

Authors: H. Froberg, H. Oettel and H. Zeller

Source: Archiv für Toxikologie, Vol. 25, 1969, Springer-Verlag Berlin, Heidelberg, New York, P. 268-295.

A. Introduction:

Not all the substances, whose possible teratogenic effect must be tested, are soluble in water, physiological salt solution and Ringer's solution. Hence, they must be applied, in the form of suspensions or emulsions according to their mode of utilization, orally or parenterally. Natural substances, beside synthetic products like carboxymethyl cellulose, are used as suspensions in manufacturing these preparations. That is why the influence of suspensions involving natural substances was tested in comparison with distilled water and Ringer's solution as to gestation in mice. This was done especially since WILLIAMSON and Collaborators (1963), as well as CARTER (1965), proved in their tests conducted on chick embryos that inert substances, like sand, ground glass, colloidal Al or talc, produced malformations with the same frequency as definitely teratogenic substances.

B. Material and Methods:

1. Animal Species Used:

Mice of the NMRI-breed were used for the tests (animals from closed random breeding; Breeder Dr. Haakh, until late 1964: Federal Research Institution for Virus Diseases in Animals, since 1965: Firma Ivanovas, Kissleg, Allgäu). Upon reception, all the mice received a protective inoculation of active vaccine virus against ectromelia at the Industrial Hygiene Pharmacological Institute of the BASF (Zeller and Reckzeh, 1965).

2. Type of the Substances Tested and of the Substance Specimens:

Table 1 contains a survey of the substances tested and substance specimens, and reveals their origins.

Table 1: Survey of the substances tested

Bezeichnung	Chargen- nummer	Herkunft	
Aqua destillata	—		
Ringerlösung	—		
Gummi arabicum	G 1012	E. Merck, AG	Darmstadt
Agar	51581	E. Merck, AG	Darmstadt
Carrageen	—	F. Schulze u. Co	Mannheim
Guar	M 175	Meyhall Chemical AG	Kreuzlingen (Schweiz)
Talkum	T 61754	E. Merck, AG	Darmstadt
persischer Tragacanth Nr. 0	—	E. Merck, AG	Darmstadt
persischer Tragacanth Nr. 1	434209	E. Merck, AG	Darmstadt
persischer Tragacanth Nr. 2	387011	E. Merck, AG	Darmstadt
persischer Tragacanth Nr. 3	263868	E. Merck, AG	Darmstadt
persischer Tragacanth Nr. 4	277520	E. Merck, AG	Darmstadt
persischer Tragacanth Nr. 5	419414	E. Merck, AG	Darmstadt
persischer Tragacanth Nr. 6	8021 65	Imhoff u. Stahl, GmbH	Mannheim
persischer Tragacanth Nr. 7	B 420, 5165	Riedel de Haen, AG	Seelze-Hannover
indischer Tragacanth Nr. 1	14009, 64	Imhoff u. Stahl, GmbH	Mannheim
indischer Tragacanth Nr. 2	562 65	Imhoff u. Stahl, GmbH	Mannheim

The terms "Persian tragacanth" designate the officinal tragacanth from the Near East. These are mucilages obtained mainly from *Astragalus gummifer* Labillardière and from related asiatic astragal species, all of them belonging to the Leguminosae family (TSCHIRSCH, 1912; BERGER, 1964). In previous time, this astragal mucus, called also "true tragacanth", was extended with "Indian tragacanth", and still today Indian tragacanth is often used as a substitute for the more expensive pure tragacanth.

Indian tragacanth, called also "false tragacanth", Karaya- or Sterculia-rubber, is obtained by scraping the bark of the trunk of *Sterculia ureaus* Roxb., a tree widely distributed in Hither India (Hindustan). Unlike true tragacanth, Sterculia-rubber contains no starch, possesses no adhesive power, and swells much more rapidly in water.

3. Nutrient Media:

Merck-Standard I-nutrient agar and Merck-Standard-nutrient broth were used in obtaining the bacteriological nutrient media. The various differential nutrient media were prepared according to the prescriptions indicated by HALLMANN ("Bakteriologische Nährböden" - Bacteriological Nutrient Media, Thieme-Verlag, Stuttgart, 1953).

4. Methode Used in Testing the Teratogenic Effect:

In teratogenic tests, the fertilization periods should be indicated as accurately as possible. Hence, in our Institute, when teratogenic tests are conducted on mice, several 100 pubescent female mice are placed together each time for 2 hours with the males (bucks) (5 females with one male), once a week from 10 to 12:00 AM. When the cohabitation is terminated, each animal exhibiting a vaginal plug is isolated. In the case of these "plug mices", the pairing day is called the 1st day of gestation (FROHBERG and OETTEL, 1964).

On the 19th day of gestation, thus one day before the normal littering day, all the treated or untreated mice are killed, and the number of the implantation places, of the intact and resorbed fetuses, as well as the number of the dead but externally uninjured fetuses, are determined. The implantation places, which can be recognized by the "metrial glands", which can be identified as quite perfused yellowish nodules on the mesometrium insertion of the uterine excrescences, and which are not occupied by living or artificially dead fetuses or fetal resorptions, characterize the number of the miscarried embryos.

The body length and weight of the fetuses are determined, then their malformations are examined macroscopically. This examination is conducted systematically from head to tail. Subsequently, the fetuses are fixed in alcohol; the soft parts are clarified in caustic potash and, in order to evaluate the individual bones, the skeletal system is dyed with alizarin red S by a modified Dawson method. These clarified and colored fetuses are kept in 100% glycerine DAB 6 (FROHBERG and OETTEL, 1966).

In order to be able to make a statement regarding the possible embryotoxic effect of a product in the case of the animal species used for the tests, one must possess an accurate knowledge of the spontaneous values. Hence investigations of the littering size, of the rates of fetal resorption and of malformation, were conducted on 413 pregnant and untreated NMRI mice.

In the uteri of 414 untreated animals, 4293 implantation places were found, that is 10.4 on an average per mother animal (See Table 2).

Table 2: Spontaneous values in 414 NMRI control-mice. The mother animals were caused to die on the 19th day of gestation.

a. Implantations b. Fetuses c. Alive d. Malformed e. Dead
f. Resorptions g. Miscarriages h. Total number i. Average per animal

	a. Implan- tationen	b. Feten lebend	c. miß- gebildet	d. runts	e. tot	f. Resorp- tionen	g. Aborte
Gesamtzahl (h)	4293	3918	60	18	20	343	12
Durchschnitt pro Tier (i)	10.4	9.5	0.14	0.04	0.05	0.83	0.03

18 Arch. Toxikol., Bd. 25

From the 4293 implanted embryos, 343 had died or had been resorbed. Thus the average quota of fetal resorption per animal was 0.83. One must bear in mind, however, that fetal resorptions were found only in 199 of the 414 control mice, and also one resorption in 120 animals, 2 resorptions in 44 mice, and more than 2 resorptions in 35 animals. One mouse, in each case, had even 6 or 8 spontaneous resorptions. On the 11th day of gestation, in a control animal, all the 12 fetuses were expelled as miscarriages.

For the most part, the mice had 9 - 13 fetuses per litter, and the 3918 living fetuses of the 414 control animals exhibited on an average a length of 2.2 cm and a body weight of 1.2 g. Eighteen living fetuses of the altogether 14 mother animals, must be regarded, with a body weight of only 0.6 - 0.8 g, as "congenital runts". In 18 mothers, altogether 20 dead, nonmalformed, "normally" developed fetuses were found in the uterus.

Figure 3: Spontaneous malformations in 3918 fetuses from 414 NMRI-control-mice. The mother animals were caused to die on the 19th day of gestation.

a. Type of malformation b. Number of malformed fetuses c. Frequency
d. Cleft palates e. Thoracic vertebral body hyplasty g. Aplasia of the
vertebrae and ribs h. Malformations of the ribs i. Out of 50 mother animals.
f. exencephaly j. microcephaly k. micrognathy

a. Art der Mißbildung	b. Zahl der mißgebildeten Feten	c. Häufigkeit (%)
d. Gaumenspalten	40	1,02
f. Exencephalie	6	0,15
j. Mikrocephalie	2	0,05
k. Mikrognathie	1	0,03
e. Brustwirbelkörperhypoplasie	1	0,10
g. Wirbel- und Rippenaplasie	1	0,03
h. Rippenmißbildungen	6	0,15
	60	1,53
	von 50 Mutter-tieren	

Out of the 3918 living fetuses, 60 (1.53%), which originated from 50 (12%) of the 414 mother animals, exhibited spontaneous malformations (Table 3), and also 40 fetuses (1%) had cleft palates. Thus they presented the most frequent spontaneous malformation in our mice-breed. Four fetuses had exencephaly, 2 fetuses had microcephaly, 1 fetus exhibited a micrognathy, and in 4 fetuses, hypoplasiae or aplasiae of some thoracic vertebrae were detected. Six fetuses had malformations of the ribs, and in one stunted animal, all the thoracic vertebrae, the ribs, and most of the lumbar vertebrae were missing.

C. Results:

1. Testing the Fetotoxic Effect of Different Suspensions:

From the 11th to the 15th day of pregnancy, gravid mother-mice received daily 0.2 ml per animal of the suspension preparations (mentioned in Table 4) by intraperitoneal injection. The individual results are shown in Table 4.

Table 4: Effects of suspensions on the pregnancy of NMRI mice 5 x 0.2 ml/animal intraperitoneally as aqueous suspension from the 11th to the 15th day of gestation (The mother animals were caused to die on the 19th day of gestation)

a. Substance b. Number of mother animals c. Total number per group with miscarried fetuses d. Implantations e. Total number per group
f. Average per mother animal g. Resorptions h. % referred to the total number of implantations i. Miscarriages j. Fetuses k. Alive l. Weight
m. Length n. Dead o. Malformed p. Type of malformation q. Distilled water
r. Ringer's solution I. Cleft palate 2. Umbilical hernia 3. Malformation of the ribs 4. Aplasia of the Os interparietale.

a. Substanz	b. Mutter- tierzahl		d. Implan- tationen		g. Resorp- tionen		i. Aborte		j. Feten				n. tot		o. mißgebildet		
	c. ges. mit miß- geb. Feten		ges. 0		h ges. %		h ges. %		k lebend		l. Ge- wicht g 0		m. Länge cm 0		ges. ges. %		p. Art der Miß- bildung
	ges.	mit miß- geb. Feten	ges.	0	h ges.	%	h ges.	%	h ges.	0	l	Ge- wicht g 0	m. Länge cm 0	ges.	ges. %		
q. Aqua dest.	21	1	225	10,7	24	10,7	0	0	200	9,5	1,2	2,2	1	1	0,4	1	1
r. Ringerlösung	5	0	54	10,8	4	7,4	0	0	50	10,0	1,3	2,5	0	0	0		
Gummi arabicum (1%)	8	1	92	11,5	3	3,3	0	0	88	11,0	1,3	2,2	1	1	1,1	1	2
Agar (1%)	9	2	94	10,4	9	9,6	4	4,3	81	9,0	1,2	2,2	0	4	4,3	3	1
																1	3
Carrageen (1%)	9	1	92	10,2	9	9,8	0	0	82	9,1	1,2	2,2	1	1	1,1	1	1
Guar (1%)	11	0	113	10,3	42	37,2	9	8,0	52	4,7	1,1	2,1	10	0	0		
Guar (1%) 5 x 0,1	8	0	72	9,0	12	16,7	2	2,8	55	6,9	1,1	2,1	3	0	0		
Talkum (1%)	9	1	87	9,7	11	12,6	0	0	76	8,4	1,2	2,3	0	1	1,1	1	2
Tragant Nr. 0 (1%)	8	0	82	10,3	44	53,7	38	46,3	0	0	—	—	0	0	0		
Gummi arabicum (10%)	18	5	202	11,2	57	28,2	0	0	145	8,1	1,2	2,3	0	6	3,0	3	1
																3	4
Talkum (10%)	14	1	128	9,1	50	39,1	0	0	72	5,1	1,2	2,2	6	2	1,6	2	1

ges. = Gesamtzahl pro Gruppe, 0 = Durchschnitt pro Muttertier, % bezogen auf Gesamtimplantationszahl. 1 Gaumenspalte
2 = Umbilicalhernie, 3 = Rippenmißbildung, 4 = Aplasia des Os interparietale.

After a 5 time intraperitoneal injection from the 11th to the 15th day of gestation of each time 0.2 ml per animal of distilled water, Ringer's solution, 1% aqueous gum arabic, agar and carrageen mucilage or of a 1% aqueous talc suspension, there was no disturbance of the pregnancy. After the injection of corresponding amounts of a 1% aqueous guar mucilage, one of the 11 mother mice died intercurrently. Forty-two fetuses were resorbed, 9 were expelled as miscarriages, and 10 were dead at the time of the killing of the mother animals

on the 19th day of gestation. In doses of 5 times 0.1 ml/animal, a 1% guar mucilage no longer influenced practically the fetal development.

After the 5 time intraperitoneal injection of each time 0.2 ml of a 1% aqueous mucilage of a commercially obtained Near East DAB 6 tragacanth, surprisingly all the fetuses were resorbed or were expelled as miscarriages. This ^{feto}embryo-toxic effect of the 1% aqueous tragacanth mucilage investigated was considerably stronger than that of a 10% aqueous gum arabic mucilage or of a 10% aqueous talc suspension.

2. Embryotoxic Effect of Persion Tragacanth:

Since the ^{feto}embryotoxic effect (observed in the first test) of the investigated tragacanth specimen stood in contrast with all the other suspensions tested, and in order to exclude experimental errors or an accidental contamination of this specimen by unknown active substances, which could have caused the embryotoxic effect, 5 other charges (No. 1 - 5) of the DAB 6-tragacanth (of Persion origin) provided by the Merck Company and used in Test 1, as well as in each case one specimen of Persion tragacanth of the Imhoff & Stahl Company (No. 6) and of the Riedel de Haen Company (No. 7) were introduced into the investigations.

In these tests the animals were injected in each case with 0.2 ml of the 1% mucilages in distilled water only on the 11th and 12th day of gestation. Furthermore, some mice were treated on the 14th and 15th day of gestation with three different Persion tragacanth specimens in the corresponding way. The individual results are given in Figure 1.

Figure 1: Embryotoxic effect of Persion tragacanth for NMRI mice 2 x 0.2 ml/animal intraperitoneally as 1% aqueous mucilage. ■ Resorptions, ▨ Miscarriages, ▩ Dead fetuses, □ Alive fetuses.

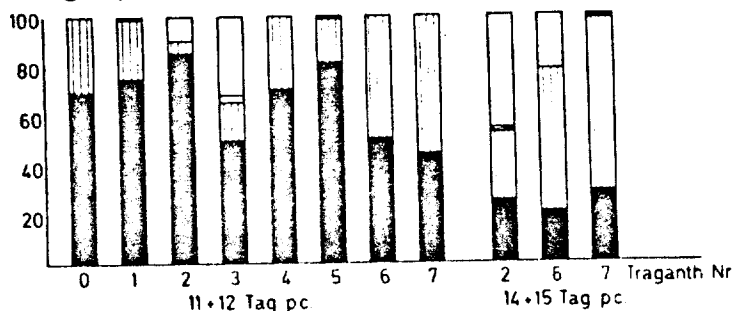


Abb. 1. Embryotoxische Wirkung persischen Tragacanth für NMRI-Mäuse 2 x 0.2 ml Tier i.p. als 1%iger wässriger Schleim. ■ Resorptionen, ▨ Aborte, ▩ tote Feten, □ lebende Feten

Five hundred and twenty eight of the 818 fetuses of the 87 mother mice, into which each time 0.2 ml/animal had been injected intraperitoneally on the 11th and 12th day of gestation, were resorbed, and 255 were expelled as miscarriages. After injection on the 14th and 15th day of pregnancy, 12 of the 160 fetuses of the 15 mother animals were dead, 40 resorbed, and 82 were expelled as miscarriages.

The miscarriages, externally recognizable through vaginal bleedings, occurred in most cases 2 to 3 days after the treatment. While animals were being killed during vaginal bleeding, dead fetus residues were found in the uteri.

Thus all the tested charges of Persian tragacanth exerted a considerable embryotoxic effect.

Since already an only two-time intraperitoneal injection of 0.2 ml each time of a 1% tragacanth mucilage of Persian origin, had a highly ^{feto}embryotoxic effect, a Persian tragacanth specimen of the three supplying Companies was injected into gravid mice only one intraperitoneally. The injections were conducted on the 11th, 12th or 14th day of gestation. See the individual results in Table 5.

Table 5: ^{feto}Embryotoxic effect of Persian tragacanth for NMRI mice 1 x 0.2ml/animal intraperitoneally as 1% aqueous mucilage (The mother animals were caused to die on the 19th day of gestation)

a. Day of application percent b. Number of mother animals c. Total number per group
d. with born fetuses e. Implantations f. Average per mother animal
g. Resorptions h. % referred to the total number of implantations i. Miscarriages
j. Fetuses k. Alive l. Weight m. Length n. Dead o. Malformed
p. Type of malformation 1 = cleft palate 2 = Kyphosis 3 = Malformation of the thoracic vertebral body 4 = Umbilical hernia 5 = Clubfoot on the left
aplasia of the metatarsals and phalanges III and IV.

Tabelle 5. Embryotoxische Wirkung persischen Traganths für NMRI-Mäuse 1 : 0,2 ml/Tier i.p. als 1%iger wäßriger Schleim (Tötung der Muttertiere am 19. Trächtigkeitstag)

Tragant Nr.	a. Appli- kations- tag p.e.	b Mutter- tierzahl		c Implan- tationen		g Resorp- tionen		Aborte		j Feten							
		e	ges. mit miß- geb. Feten	f ges.	o	f ges.	%	ges.	%	K	lebend	n, tot		d mißgebildet		p, Art der Miß- bildung	
												ges.	o	Ges. M wicht g o	Länge cm o		ges.
2	11	8	3	85	10,6	11	12,9	0	0	72	9,0	1,1	2,1	2	6	7,1	6 - 1
6	11	10	0	104	10,4	58	55,8	13	12,5	27	2,7	1,0	2,1	6	0	0	
7	11	6	0	64	10,7	46	71,8	9	14,1	9	1,5	1,2	2,3	0	0	0	
0	12	15	5	150	10,0	45	30,0	0	0	105	7,0	1,2	2,2	0	10	6,7	9 - 1 1 2
2	12	11	5	116	10,5	17	14,7	5	4,3	93	8,5	1,1	2,2	1	14	12,1	13 - 1 1
6	12	8	2	81	10,1	55	67,9	0	0	22	2,8	1,0	2,0	4	3	3,7	3 - 1
7	12	8	0	84	10,5	22	26,2	62	73,8	0	0	0	0	0	0	0	
0	14	5	0	45	9,0	41	91,1	4	8,9	0	0	0	0	0	0	0	
2	14	3	2	30	10,0	5	16,7	0	0	24	8,0	1,1	2,2	1	3	10,0	3 - 1
6	14	8	2	90	11,3	20	22,2	0	0	22	2,8	1,0	2,1	48	7	7,8	5 - 1 1 1 1 - 2
7	14	5	0	52	10,4	0	0	31	59,6	4	0,8	1,0	2,1	17	0	0	

ges. = Gesamtzahl pro Gruppe, o = Durchschnitt pro Muttertier, % = bezogen auf Gesamtimplantationszahl, 1 = Gaumenspalte
2 = Kyphose, 3 = Brustwirbelkörpermißbildung, 4 = Umbilicalhernie, 5 = Klumpfuß links Aplasie der Metatarsalia und Phalange III und IV.



Abb. 2. Links: Fet mit hypoplastischer Fehlbildung der hinteren Extremität und Tibiaaplasie, Behandlung der Muttermaus: einmal 0,2 ml pro Tier i.p. eines 1%igen wäßrigen Schleims aus persischem Traganth am 12. Trächtigkeitstag. Rechts: Normaler Kontrollfet, Muttermäuse wurden am 19. Trächtigkeitstag getötet, Aufhellung und Anfärbung des Skelettsystems nach einer modifizierten Dawson-Methode

In these tests, the strongest ^{feto}embryotoxic effect was exerted by Charge No. 7, but even after injection of the tragacanth-Charge No 6 on the 11th or 12th day of gestation, the majority of the fetuses were resorbed or expelled as miscarriages.

After the injection of this tragacanth (No. 6) on the 14th day of gestation, 20 fetal resorptions, 22 alive and 48 dead fetuses were found in the uteri of the 8 mother mice treated. One of the 22 living, and 4 of the 48 dead fetuses had cleft palates. Furthermore, kyphosis was detected in a dead fetus, and in another a malformation of the thoracic vertebral body. The seven malformed living and dead fetuses originated from two of the eight mother mice.

The Persian tragacanth specimens No. 0 and 2, used for these tests, produced fetal resorption and abortions with lower frequency; nevertheless, 29 of the 294 living fetuses and 2 of the dead ones had cleft palates. The 10% proportion of cleft palates is 10 times higher than the spontaneous cleft palate frequency (1.02%) of our NMRI mice-breed. The 29 living and 2 dead fetuses with cleft palates come from 14 different mother animals.

Cleft palates occurred most frequently in the mother mice treated on the 12th day of pregnancy with Persian tragacanth mucilage No. 0 and No. 2; then in 10 of the 26 mother animals, fetuses (21 living and 1 dead) were found with a fissure of the palate. Furthermore, one fetus of these 10 mother animals had an ectopia of the abdominal organs. In another fetus with uranoschism, once the skeleton had been colored, a hypoplastic malformation of the right rear extremity with tibial aplasia was detected (Figure 2). Thus altogether in 10 of the ²⁶ mother mice treated on the 12th day of gestation, 23 living fetuses, and one dead, were found with malformations.

Figure 2: Left: Fetus with hypoplastic malformation of the rear extremity and tibial aplasia. Treatment of the mother mouse: one 0.2 ml per animal intraperitoneally of a 1% aqueous mucilage from Persian tragacanth on the 12th day of gestation. Right: Normal control fetus. The mother mice were caused to die on the 19th day of pregnancy. Clarification and coloration of the skeletal system by a modified Dawson method.

A 1% aqueous mucilage of Persian tragacanth of different origin(s) exerted an embryotoxic effect not only after the repeated, but already after the single intraperitoneal injection of 0.2 ml per animal in the middle of the gestation in the case of the NMRI mice used for the investigations.

In order to determine whether tragacanth from other regions of cultivation possesses the same embryotoxic effect as Persian tragacanth, two different specimen of Indian tragacanth (No. 1 and 2) were tested in the same way as in Experiment 1.

Unlike Persian tragacanth, these Sterculia-rubber specimens, even after the five-time injection of 0.2 ml per animal from the 11th to the 15th day of gestation - a dose which in the case of the Persian tragacanth always caused 100% fetal death - failed to affect the fetal development of the mice. Only the proportion of resorptions was slightly raised as compared with the normal occurrence, and 7 of the 239 fetuses (2.9%), from 7 different mother animals, had uranochism.

The repeated five time subcutaneous injection of each time 0.2 ml per animal from the 11th to the 15th day of gestation of a 1% aqueous mucilage, obtained from Persian tragacanth No. 2, failed to affect the fetal development of all the 12 mother mice.

Hence, it must be assume that the embryotoxic effect (observed after intraperitoneal injection) of different tragacanth charges of Persian origin is conditioned not by a systemic, but by a direct action on the uterus.

Since some of the investigated suspensions are used also in manufacturing suspensions to be orally applied, and since tragacanth as well as guar and agar are allowed as food thickeners, the fetotoxic effect of these natural substances, as compared with gum arabic and talc, was tested by oral application also on 35 mice. The soundings (probings) were undertaken from the 11th to the 15th day of gestation daily in amounts of 0.5 ml per animal in the form of 1% and 10% suspensions or mucilages. Despite this high dosage, the proportion of fetal resorption, the average number of living fetuses,

their body lengths and weights as well as the malformation rate in the tests with Persian and Indian tragacanth, agar and gum arabic, lay within normal limits. It is only after the sounding of 10% talc suspension and of 1% guar mucilage that the proportion of fetal resorption was raised.

Thus Persian tragacanth in oral application of large amounts even, like the other natural substances tested, failed to affect the fetal development of the NMRI mice.

3. Embryotoxic Effect of Suspensions in Early Pregnancy:

Previous tests with chemical, teratogenic compounds like formamide, monomethyl formamide, 1-ethylene imino-2-oxybutene-3 and 6-mercaptopurine showed that during the early period of gestation the mice-embryos were only slightly sensitive to teratogenic noxas (OETTEL and FROHBURG; 1964/1965). Hence tests were conducted in order to determine whether the embryotoxic effect of Persian tragacanth after intraperitoneal injection during the first third of the gestation is weaker than after application between the 11th and 15th day of gravidity.

Sixteen mice were injected from the 4th to the eighth day of gestation with 0.2 ml of a 1% aqueous mucilage from Persian tragacanth intraperitoneally. For comparison purpose, mice were treated in the same way with distilled water or mucilages from different natural substances (agar, guar, gum arabic, Indian tragacanth). Like in the previous tests, the animals were caused to die on the 19th day of gestation, that is one day before the normal littering term.

In all the 31 mice treated with distilled water, agar, gum arabic or Indian tragacanth - apart from the 17.6% amount of resorption some what increased in the "agar animals" as compared with what is normal - the fetal development was not disturbed. The number of fetuses, their weight and length, the proportion of malformations and resorptions corresponded to those of the untreated mice. On the other hand, normally developed fetuses were found only in one of the 16 animals treated with Persian tragacanth. The uteri of the other 15 mother mice, when these were killed on the 19th day of gestation did not differ from those of nonpregnant animals.

The same dose, administered on the 6th and 7th day of gestation, caused vaginal bleedings in 3 of the 7 mother animals. Hence, these mice, and also those treated on the 7th and 8th day of gravidity, were caused to die not one day before littering, but already on the 12th day post coitum. At this moment, fetuses (66) still living in the uteri were found only in 6 of the 13 mice. In 4 of the 13 mothers, all the fetuses had been expelled. It was possible, however, to determine the number of the expelled embryos by observing the places of implantation that were still visible - in the form of "metrial glands".

On the other hand, in 6 out of 16 mice, which had been treated from the 4th to the 8th or from the 4th to the 7th day of gestation with tragacanth mucilage and had been killed immediately, the uteri did not differ from those of nonpregnant mice. Since, however, in 2 of the 16 animals, normal embryos, corresponding in their development to the 7th or 8th day of gestation, were present in the uteri, and since in the other mice the places of implantation were clearly recognizable on the mesometrium insertion, it must be assumed that in the 6 animals without "metrial glands" the embryos were expelled already before, or at the beginning of, ^{the} embedding process.

Thus the embryotoxic effect of Persian tragacanth was weaker in the case of injections administered during early gestation than in application in the middle of the gravidity period. As is shown by the tests, the mice-embryos with increasing age become more sensitive to the embryotoxic effect of Persian tragacanth; whereas Persian tragacanth mucilage injected on the 4th and 5th day of gestation failed to disturb the embryo development, fetal death and miscarriage or resorption occurred after injection on the 6th and 7th day of gestation, and still more markedly after application on the 7th and 8th day of gravidity.

4. Comments on the Problem of the Antineoplastic Effect of Suspensions:

ROE (1959), GALBRAITH, MAYHEW and ROE (1962) as well as MAYHEW and ROE (1964) found that native Persian tragacanth, but not heated Persian tragacanth and Karaya rubber (= Indian tragacanth) inhibited the growth of mice-ascites-tumors.

Tabelle 7. Antineoplastische Wirkung von Naturstoffen am Ehrlich-Ascites-Carcinom der Maus. 5 · 0,2 ml/Tier i.p. einer 1%igen wäßrigen Suspension (Montag bis Freitag). (Erste Injektion 2 Std nach der Tumortransplantation)

a, Substanz	b, Lebensdauer in Tagen	c, Ascites- menge beim Tod (g)	d, Tumor- hemmung in %	
	e, Mittel- wert	f, Absolute Streuung „range“		
<i>Versuchsreihe I</i>				
Unbehandelt	11,9	8—15	9,6	—
Aqua dest.	11,5	7—15	9,1	4,4
Gummi arabicum 10%	8,2	6—12	4,1	57,6
Talkum 10%	9,1	7—12	5,2	45,3
Ind. Traganth (Nr. 2) 1%	9,0	8—15	5,0	47,5
Pers. Traganth (Nr. 0) 1%	6,8	4—8	1,6	83,2
<i>Versuchsreihe II</i>				
Unbehandelt	14,9	12—18	9,3	—
Aqua dest.	12,8	7—17	8,0	13,7
Gummi arabicum 10%	11,3	9—14	5,1	45,1
Talkum 10%	10,7	4—15	4,4	52,5
Ind. Traganth (Nr. 1) 1%	10,2	8—13	4,2	54,4
Pers. Traganth (Nr. 0) 1%	8,1	5—14	0,5	94,7
<i>Versuchsreihe III</i>				
Unbehandelt	11,6	9—15	6,2	10,5
Agar (Fäden) 1%	9,1	8—13	2,0	67,7
Carrageen 1%	11,0	8—16	5,6	10,5
Guar 1%	9,1	8—12	2,8	55,2

Bei der Tumortransplantation wurde den Mäusen jeweils 1 ml verdünnter, frischer Ascites i.p. injiziert. Zur Verdünnung wurde Ringerlösung verwandt. Das Verdünnungsverhältnis betrug in der Versuchsreihe I 1:1, in den Versuchsreihen II und III 1:10.

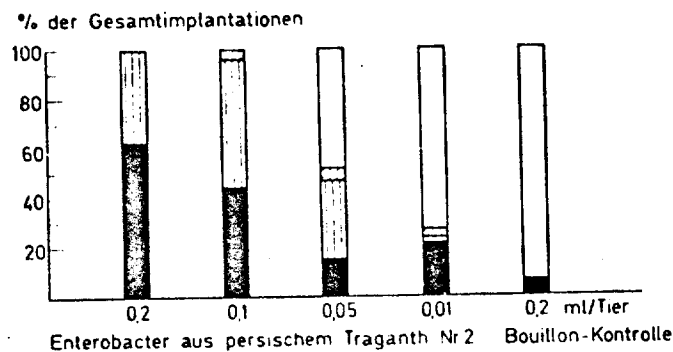


Abb. 5. Embryotoxische Wirkung kleinerer Dosen von Enterobacter-Bouillon-Sterilfiltraten für NMRI-Mäuse einmal i.p. am 12. Tag p.c. ■ Resorptionen, □ Aborte, ▨ Mißbildungen, □ lebende Feten

On the basis of mitosis counts on the Landschütz-ascites-tumor of the mice, MAYHEW and ROE (1964) surmized that the cause of the antineoplastic effect (observed by them) of the native true tragacanth was a mitosis inhibition and an "active component" of this drug, influencing the surface of the tumor cells. Hence tests were conducted in order to determine whether the embryotoxic effect, observed in the case of the Persian tragacanth, was an expression of the cytotoxic action described by ROE and Collaborators for tragacanth. For this purpose, Persian tragacanth (No. 0) was subjected to experimentation regarding antineoplastic action on the Ehrlich-ascites-carcinoma of the mouse, as compared with other suspensions and natural substances.

For each series of tests, 40 to 60 mice were used. All the animals were inoculated intraperitoneally with fresh ascites 1:1 or 1:10 diluted with Ringer's solution. Each test or control group comprised 10 mice; the controls remained untreated. All experimental animals - starting two hours after the tumor transplantation - were inoculated intraperitoneally on 5 consecutive days with 0.2 ml per animal of distilled water or of a 1% aqueous suspension of the corresponding suspensions. The mice were weighed daily, they were observed until their spontaneous death; then they were dissected. The ascites was removed by sponging the abdominal cavities with cellulose, and its amount was determined by subsequently weighing the animals. The evaluation of the antineoplastic effect was made on the basis of the ascites amount found, and also of the surviving period of the tumor animals (OETTEL and WILHELM, 1957). See individual results on Table 7.

Table 7: Antineoplastic effect of natural substances on the Ehrlich-ascites-carcinoma of the mouse. Administration of 5 x 0.2 ml/animal intraperitoneally of a 1% aqueous suspension (Monday until Friday). (First injection two hours after the tumor transplantation).

a. Substance	b. Life duration in days	c. Average value	d. Absolute
range of dispersion	e. Amount of ascites at death	f. Tumor inhibition in %	
g. Test series I	h. Untreated	i. Distilled water	j. Gum arabic 10%
k. Talc 10%	l. Indian tragacanth	m. Persian tragacanth	o. agar (threads)
p. Carrageen	q. Guar		

In the tumor transplantation, 1 ml of diluted fresh ascites was injected intraperitoneally into the mice. Ringer's solution was used for dilution. Dilution ration 1:1 in the series of tests I, and 1:10 in II and III.

With the exception of the carrageen animals, in all the series of tests, in the mice treated with the different suspensions, the ascites amount decreased by 50% as compared with the controls, and even by 80 to 90% in the animals treated with Persian tragacanth. This decrease of the ascites amount, however, implied in every case the shortening of the surviving period of the experimental animals as compared with the controls, and thus does not express a true tumor inhibition.

5. Effects of Heat Sterilization and of Antibiotics on the Embryotoxic Action of Persian Tragacanth:

In trying to find the causes of the embryotoxic effect of Persian tragacanth, tests were performed in order to ascertain whether this effect was due possibly to microbial contaminations. For this purpose, a specimen of Persian tragacanth (No. 2) and for comparison a specimen of Indian tragacanth (No. 1) were dry-sterilized 30 minutes at 170 C. Then, from these two tragacanth specimens and with distilled water in each case a 1% mucilage was manufactured which was used in treating intraperitoneally mice from the 11th to the 15th day of gestation. Furthermore, mother mice were treated in the same way with an aqueous mucilage from Persian tragacanth which had been previously incubated for 48 hours at 37 C after adding penicillin (100 I.U./ml) and streptomycin (100 µg/ml) (See Figure 3).

Figure 3: Embryotoxic effect of pretreated Persian tragacanth for NMRI mice 5 x 0.2 ml/animal intraperitoneally as 1% aqueous mucilage from the 11th to the 15th day p.c.(?) ~~Resorptions~~ Miscarrages ~~Dead~~ fetuses ~~Living~~ fetuses
a. % of the total implantations b. Untreated c. Antibiotics d. Sterilized

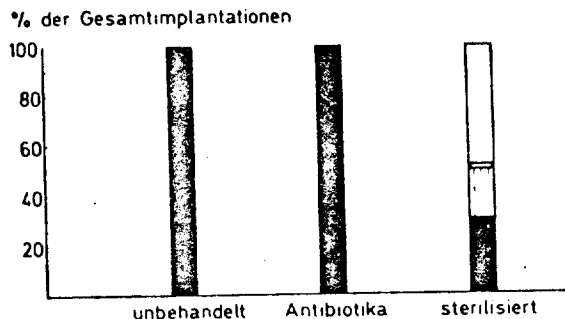


Abb. 3. Embryotoxische Wirkung vorbehandelten persischen Tragacanth für NMRI-Mäuse 5 x 0.2 ml Tier i.p. als 1%iger wässriger Schleim vom 11. - 15. Tag p.c.
■ Resorptionen, ▒ Aborte, ░ tote Feten, □ lebende Feten

Through a 30 minute sterilization at 170 C, the embryotoxic effect of Persian tragacanth was decreased, since even after the five time injection of this mucilage, manufactured from sterilized true tragacanth, only half the fetuses were resorbed or were expelled as abortions. As was expected, heat-sterilized Indian tragacanth influenced the fetal development just as little as untreated (Indian tragacanth).

A penicillin and streptomycin addition failed to lower the embryotoxic effect of Persian tragacanth.

6. Bacteriological Investigation of the Different Tragacanth Specimens:

Since it was possible to lower the embryotoxic action of Persian tragacanth through heat sterilization, it was presumable that microorganisms or their products of metabolism might be the cause of the embryotoxic effect of Persian tragacanth. Hence, the different charges of Persian and Indian tragacanth were examined bacteriologically.

In order to isolate the microorganism contained in the individual tragacanth charges, 1% mucilage specimens were prepared under sterile conditions with sterile physiological salt solution. From these specimens, after a 48 hour incubation at 37 C, a loop was inoculated on nutrient agar and the dishes were incubated at 37 C for 24 to 48 hours. Furthermore from the different 1% mucilage specimens, 1 ml in each case was placed in 50 ml of nutrient broth, and thus was processed in the same way.

In order to determine the presence of possible impurities through fungi, the same tragacanth specimens were inoculated also on Sabouraud nutrient media and Sabouraud broth, and were incubated for 14 days at 22 C.

From the germs, grown on the agar dishes and in the broth cultures, pure cultures were obtained, and the different types of germs were examined morphologically, biochemically, and in the case of the gram negative bacteria serologically also (specimen agglutination with salmonella cumulative serum I-Behring Plant).

In order to obtain an accurate determination of the isolated gram negative germs, the following properties were tested regularly, or the following reactions were conducted:

Motility

Hemolysis

Gelatin liquefaction

H₂S formation

Urea dissociation (splitting)

Methyl red reaction

Voges-Proskauer reaction

Indole reaction

Koser citrate

"Multicolored Series" with the following hydrocarbons and alcohols: dextrose, lactose, saccharose, salicin, inositol, adonitol, dulcitol.

For further differentiation, in individual cases, the following culture characteristics were investigated also:

Splitting of sorbitol, xylose, arabinose, starch.

Behavior in litmus milk, nitrate reduction, detection of

Phenylalanine-decarboxylase, lysine-decarboxylase, catalase, cytochrome-oxydase.

In the case of the gram positive bacilli, the following culture characteristics were used for diagnostic purpose:

Growth in broth and on agar

Motility

Hemolysis

Gelatin liquefaction

Splitting of dextrose, lactose, saccharose, xylose, mannitol

Indole reaction

Voges-Proskauer reaction.

In the case of the isolated gram positive diplococci, the following investigations were conducted:

Growth in broth and on agar

Hemolysis

Splitting of dextrose, saccharose, lactose, mannitol, maltose, salicin, sorbitol, induline, aesculine

Indole reaction.

On the basis of the undertaken investigations, the germs, isolated out of the different tragacanth specimens, were determined as follows: Results in Table 8.

Table 8: Bacteriological findings relative to different tragacanth specimens

a. Tragacanth specimen b. Yeasts c. Fungi

c. Traganth- probe	b. Bakterien		c. Hefen Pilze
	gramnegativ	grampositiv	
<i>Pers. Traganth</i>			
1	Enterobacter aerogenes	—	—
2	Enterobacter aerogenes	—	—
3	Enterobacter aerogenes	—	Penicillium
4	Enterobacter aerogenes	—	—
5	Enterobacter aerogenes	—	—
6	Enterobacter aerogenes Flavobact. rhenanus	Bac. circulans Bac. subtilis Diplococcus (nicht differenziert)	Mucor
7	a) Enterobacter aerogenes b) Enterobacter aerogenes	Bac. megatherium Diplococcus (nicht differenziert)	—
<i>Ind. Traganth</i>			
1	—	Bac. mesentericus Bac. subtilis	— —
2	—	Bac. mesentericus Bac. subtilis	—

After these investigations, enterobacter aerogenes germs were detectable in all the Persian tragacanth, and even as pure culture in specimens 1 to 5.

In the tragacanth specimen No. 6, a flavobacterium rhenanus, belonging to the family of the achromobacteraceae, was still isolated. In the tragacanth specimens

No. 6 and 7, it was possible to detect, in addition to enterobacter-aerogenes germs and the flavobacterium, gram positive aerobic sporiferous (bacteria ?) as well as gram positive diplococci, which were not more closely differentiated. In the tragacanth specimens No. 3 and 6, a penicillium or a Mucor germ was detectable; the latter was not included in the further investigations.

In contrast with the results of bacteriological investigations, in the Persian tragacanth specimens of different origins, no germs had grown in the parallel test with the two Indian tragacanth after an incubation of 24 hours. It was only after a storage of 36 to 48 hours at + 37 C, that on the nutrient agar dishes different gram positive aerobic sporiferous bacteria (*Bac. subtilis*, *Bac. mesentericus*) were detected. The only low number of bacteria in the *Sterculia* rubber should be attributed to the fact that the commercial product is solubilized through the autoclave (Merck Index, 8th Edition 1968).

According to the investigations conducted so far, the gram negative germs contained in the Persian tragacanth specimens might be regarded as the cause of the embryotoxic effect. Hence, tests were performed in order to determine the possibility of isolating these gram negative bacteria from the abdominal cavity, after repeated intraperitoneal tragacanth injection.

For this purpose, two mice, which had been treated intraperitoneally one, 3 or 5 times with 0.2 ml of a 1% mucilage of Persian or Indian tragacanth, were caused to die nearly 8 hours after the last injection; the abdominal cavity was opened under sterile conditions, the peritoneal fluid between the partly adherent intestinal loops were removed, and inoculated on agar dishes.

It was possible to isolate the enterobacter germs, detected bacteriologically in the different Persian tragacanth specimens - although these germs were few - from the abdominal cavity of the mice treated intraperitoneally 4 or 5 times with these mucilage specimens. In the test with Persian tragacanth No. 6, the gram positive diplococci were found again in the abdominal cavity of the animals treated with this tragacanth. Nevertheless, it was impossible to isolate the gram positive aerobic sporiferous bacteria, primarily detected in the Persian tragacanth specimens No. 6 and 7 or in the Indian tragacanth No. 1, from the

abdominal cavity of the mice treated repeatedly intraperitoneally in the case of these specimens.

7. Embryotoxic Effect of Products of Metabolism of Bacteria from Persian Tragacanth:

In order to exclude a bacterially induced peritonitis as cause of the embryotoxic effect of Persian tragacanth, tests were conducted in order to ascertain whether products of metabolism of the isolated germs can also exert an embryotoxic effect. For this purpose, normal nutrient broth was inoculated with pure cultures of the gram negative germs isolated from the different Persian tragacanth specimens (2 Pt loops of a 24-hour culture on 50 ml of broth) and was incubated during 48 hours at +37 C. Then these broth cultures were passed under sterile conditions through bacteria-proof filters (Seitz/Filter - Layer EKS II (EKS = sterilization layer)). After verifying the sterility by preparing smears on agar dishes, these broth cultures made sterile by filtration were injected under sterile conditions with the products of metabolism of the enterobacter strains in mice intraperitoneally - starting on the 11th day of gestation - in doses of 0.2 ml per animal (Figure 4).

Figure 4: Embryotoxic effect of broth cultures, filtrated under sterile conditions, of enterobacter strains for NMRI mice. 1 x 0.2 ml/animal intraperitoneally on the 11th day of gestation

Resorptions,	Miscarriages,	Dead fetuses,
Living fetuses,	Malformations.	

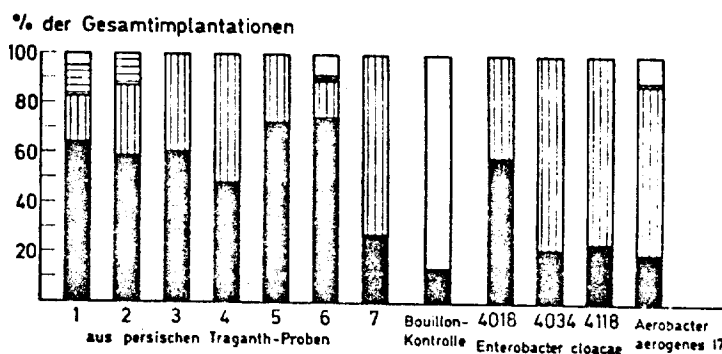


Abb. 4. Embryotoxische Wirkung steril filtrierter Bouillon-Kulturen von Enterobacter-Stmmen fr NMRI-Muse. 1 x 0,2 ml/Tier i.p. am 11. Tag p.c. ■ Resorptionen, □ Aborte, ▨ tote Feten, ▤ lebende Feten, ▧ Mibildungen

Already in the course of 12 to 15 hours after the first intraperitoneal injection, vaginal bleedings - which must be regarded as sign of miscarriage - developed in 7 of the experimental groups, which had been treated with enterobacter broth culture filtrates from the Persian tragacanth specimens No. 1 to 7, in the majority of the mice. Hence, the mice of these 7 experimental groups, which for a certain time (1 - 2 days) exhibited a ruffled skin and retracted flanks, were treated only once. Three mother animals died intercurrently.

Despite the only single intraperitoneal injection of the Persian tragacanth specimens No. 1 - 7, living fetuses were found in uteri only in 2 of the 55 mother mice treated. All the other 543 fetuses had been resorbed or had been expelled as abortions.

The 13 mice of the two other experimental groups developed no vaginal bleedings, hence they were treated from the 11th to the 15th day of gestation. In this experimental setup, the two sterile filtrates, which contained products of metabolism of the flavobacterium rhenanus and of an enterobacter aerogenes, exerted an embryotoxic effect, since about half the fetuses of the mother animals were resorbed or expelled as miscarriage. The embryotoxic action of the products of metabolism of this flavobacterium rhenanus and of this enterobacter strain, however, was considerably weaker than that of the other enterobacter strains, which already after the single injection practically caused the death of all the fetuses. In its cultural properties also, the enterobacter germ differed from those of the other enterobacter strains.

These investigations reveal that the sterile filtrates of broth cultures, inoculated with enterobacter germs and incubated for 48 hours at +37 C, exerted an embryotoxic effect.

In order to determine whether other strains of the same genus had an embryotoxic effect, sterile filtrate of broth cultures of the aerobacter aerogenes strain 17 (Dr. F. Selenka) and of 3 enterobacter cloacae strains (Dr. B. Schmidt) were tested (The long footnote at this point merely expresses thanks to these two university lecturers).

The sterile filtrates of the enterobacter strains defined caused the death of 241 out of 253 fetuses of the ³⁴ mother mice, after a single injection on the 11th day of gestation (See Figure 5). ^{P. 14a}

A 1% aqueous mucilage from Persian tragacanth, after repeated subcutaneous injection, had no embryotoxic effect. Hence, a broth culture made sterile by filtration was prepared from the same gragacanth charge in the way described above. This filtrate was injected under sterile conditions subcutaneously in 18 mice from the 11th to the 15th day of gestation in doses of 0.2 ml/animal. Furthermore 5 gravid mice were treated in the corresponding way with a broth culture made sterile by filtration; this filtrate had been prepared with enterobacter cloacae 4034. The 8 control mice received individually 0.2 ml of empty broth.

The fetal development of the 23 mother animals, treated with sterile filtrates of broth culture (obtained from Persian tragacanth No. 2 and from enterobacter cloacae 4034), did not differ from the 8 control mice treated with empty broth.

These "subcutaneous tests" corroborated the assumption that the embryotoxic effect (observed after intraperitoneal injection in NMRI mice) of tragacanth contaminated with bacteria of the genus enterobacter, is due not to a systemic but to a direct action on the uterus and on its vacular system.

In the tests reported at the beginning of this study, a single intraperitoneal injection of a 1% Persian tragacanth mucilage was followed, especially on the 12th day of gestation by increased malformations and especially by cleft palates (See Table 6). Hence, tests were conducted in order to determine whether the products of metabolism of enterobacter in doses, which practically no longer killed all the fetuses or produced resorption or miscarriage, possibly caused increased malformations.

For this test, a sterile filtrate, which had been obtained from a broth incubated for more than 48 hours with enterobacter strain 2, was injected in gravid mice in doses of 0.2; 0.1; 0.05 and 0.01 ml per animal once on the 12th day of gestation.

Like in the previous tests, on the 11th day of gestation, after a single intraperitoneal injection on the 12th day of gestation of 0.2 ml/animal of the sterile filtrate of broth culture, practically all the fetuses were resorbed or were expelled as miscarriage. The two surviving fetuses of the "0.1 ml group" had uranoschism. After the injection of 0.05 ml/animal, about half of the fetuses were killed, and even after the administration of only 0.01 ml/mouse still 16 of the 75 fetuses were resorbed, and 2 were expelled as miscarriage. Four of the 79 living fetuses of these two series of tests had malformations, especially cleft palates and some malformations of the extremities with extended subcutaneous bleedings.

In order to exclude the possibility for products of metabolism of the gram positive germ found in the different tragacanth specimens to possess an embryotoxic action, broth cultures made sterile by filtration were prepared under the same conditions as in the case of the gram negative germs from the different gram positive aerobic sporiferous bacteria (*Bac. mesentericus* and *subtilis*) and diplococci, which had been isolated from Persian and Indian tragacanth; and these cultures were used in injecting intraperitoneally mice from the 11th to the 15th day of gestation with 0.2 ml/animal daily.

In the same way, sterile filtrates of broth cultures of different other germs, stemming from the collection of the Industrial Hygiene Pharmacological Institute, such as *E. coli* I, *Staph. aureus* SG 511, *Bac. subtilis* and *Bac. mesentericus*, were tested.

Neither the sterile filtrates of broth cultures of the gram positive germs isolated from Persian and Indian tragacanth, nor the concomitantly tested control filtrates of *E. coli*, *Staph. aureus* SG 511, *Bac. subtilis* and *Bac. mesentericus* possessed an embryotoxic action on the 103 gravid NMRI mice used for these tests.

8. Effect of the Use of the Autoclave on the Embryotoxic Action of Sterile Filtrates of Enterobacter Broth Cultures:

In order to find out whether through the use of the autoclave the embryotoxic action of products of metabolism contained in the sterile filtrates of broth cultures of enterobacter strains is decreased or eliminated, sterile filtrates of broth cultures of the strains *Aerobacter aerogenes* strain 17, *Enterobacter cloacae* 4034, *Enterobacter* strain from Persian tragacanth No. 2 and *Enterobacter* strain from Persian gragacanth No. 6 were subjected to a single treatment for 15 minutes at 120 C in the autoclave. These sterile filtrates of broth culture, thus treated, were injected once daily intraperitoneally in gravid mice once 0.2 ml/ animal on the 11th day of gestation or from the 11th to the 15th day of gestation in amounts of 0.2 ml/ animal (See Figure 6).

Figure 6: Embryotoxic effect of broth cultures (obtained by sterile filtration) from *Enterobacter* strains after the use of the autoclave for NMRI mice.

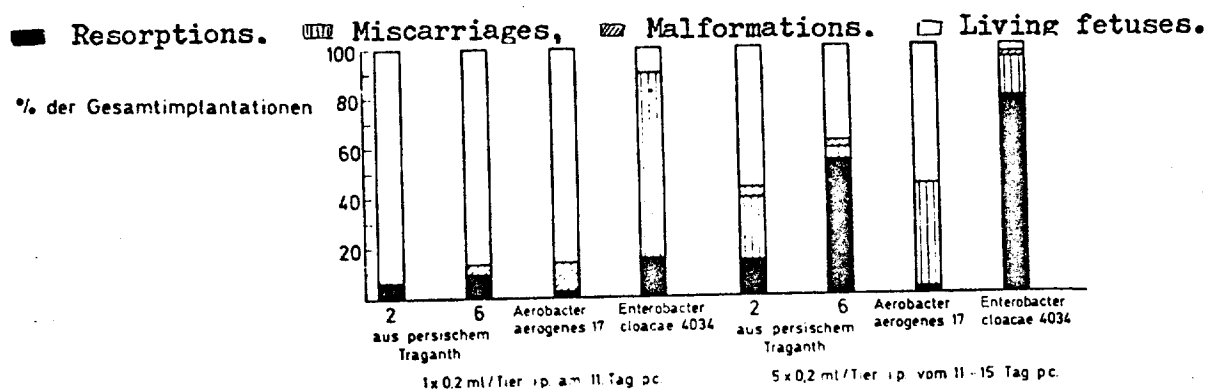


Abb. 6. Embryotoxische Wirkung steril-filtrierter Bouillon-Kulturen von *Enterobacter*-Stämmen nach Autoklavierung für NMRI-Mäuse. ■ Resorptionen, ▨ Aborte, ▩ Mißbildungen, □ lebende Feten

The use of the autoclave for 15 minutes weakened the embryotoxic effect of the products of metabolism of the different enterobacter strains, but failed to suppress it; after the five time intraperitoneal injection, about the half of the fetuses of the mother animals treated were resorbed or were expelled as miscarriages. The autoclaved sterile filtrate of the broth culture from *Enterobacter cloacae* 4034 was just as embryotoxic as the corresponding nonautoclaved sterile filtrate, since after the single injection on the 11th gestation day 77 out of 104 fetuses were expelled as abortion and 16 were resorbed.

Discussion:

Distilled water, Ringer's solution, the 1% mucilages of gum arabic, carrageen or guar, used in manufacturing suspensions or emulsions, and even 1% aqueous talc suspensions do not influence the fetal development of NMRI mice after a single intraperitoneal injection, or after several, in the sensitive phase of gestation. Under the same experimental conditions, however, once mother mice have been treated with 1% aqueous mucilages from 8 different Persian tragacanth specimens, the death and subsequent resorption or expulsion of all the fetuses occur. On the other hand, mucilage from 2 Indian tragacanth specimens exerts no effect, like the other natural substances tested, on the fetal development.

The mice-fetuses most easily injured are those involved in the treatment administered in the middle of the gestation period (11th and 12th day of gravidity). In the stage of advanced organogenesis, the fetuses become increasingly more resistant to the embryotoxic effect of Persian tragacanth. Thus the sensitivity of mice-embryos to pure tragacanth, during their development, is just as different as to known chemical or physical teratogenic noxa (WILSON, 1965; RUGH and GRUPP, 1959 as well as OETTEL and FROHBERG, 1965, 1965).

In all the mother mice, treated with a 1% aqueous Persian tragacanth mucilage intraperitoneally, dissection revealed inflammatory changes in the abdominal cavity. Hence at first it was assumed that the peritonitis, produced by the intraperitoneal injection, was the cause of the fetal injury, especially other natural substances, which do not affect the fetal development, like the investigated Indian tragacanth-, agar, or carrageen specimens which even after repeated intraperitoneal injection in the form of 1% aqueous mucilages do not exert a stimulating effect. There is an objection, however, against peritonitis as cause of the embryotoxic effect of Persian tragacanth: 1% aqueous gum arabic and especially talc suspensions after repeated intraperitoneal injection in mice cause stronger inflammatory stimulations in the abdominal cavity than Persian tragacanth, although these suspensions have just as little effect on the fetal development as Ringer's solution or distilled water. Even through five time intraperitoneal injection of a 10% aqueous gum arabic or talc suspension, the fetal development, despite most severe peritonitic inflammations and massive adherences of the ingesta in the mother animals is less disturbed than through

a single injection of 1% mucilage from Persian tragacanth. Hence, the peritoneal stimulation produced by this ~~t~~ragacanth after intraperitoneal injection should not be the cause of its embryotoxic action.

In contrast with the intraperitoneal tests, Persian tragacanth mucilage in oral application even after repeated administration of large amounts, affects the fetal development of mice just as little as the other tested natural substances. Hence - as far as a posteriori conclusions can be drawn from animal tests and applied to human beings - there should be no hesitation, from the embryotoxic effect standpoint, to continue the utilization of Persian tragacanth, beside other natural substances, as an addition to food.

A cytotoxic effect, described by ROE (1959), GALBRAITH, MAYHEW and ROE (1962) as well as MAYHEW and ROE (1964) in the case of Persian tragacanth on the basis of investigations with some mice-ascites-tumors, must be excluded, according to the investigations presented here, as cause of the observed embryotoxic effect of pure tragacanth, since the Persian tragacanth specimens, that we tested, like the other natural substances, possess on the Ehrlich ascites carcinoma of the mouse no significant antineoplastic effect after intraperitoneal injection.

According to our investigations, it is rather bacteria or their products of metabolism that must be made responsible for the embryotoxic effect of the Persian tragacanth charges tested. This is shown already by the decrease of the embryotoxic action of aqueous mucilage from Persian tragacanth after sterilization, and especially by the presence of gram negative coccoid rods of the genus *Enterobacter* in all the⁷ investigated Persian tragacanth specimens, which are not found in the two Indian tragacanth specimens. The considerable lack of bacteria in Sterculia-rubber should be attributed to the solubilization of this commercial product through the autoclave (Merck Index, 1968). The products of metabolism of the individual isolated *Enterobacter* strains, injected intraperitoneally in the form of sterile filtrates of broth cultures in gravid animals, exert a higher embryotoxic effect than the original tragacanth mucilage. The products of metabolism of bacteria of these *Enterobacter* strains should represent thus the real active form. The control tests, conducted with defined *Enterobacter* strains, show that the embryotoxic effect is specific for the bacteria products of metabolism of the isolated bacteria genus (*Enterobacter*).

21

The gram positive aerobic sporiferous bacteria and diplococci, isolated from Persian tragacanth, generate no products of metabolism with embryotoxic action. In the same way behave also the gram positive germs, which were isolated from the Indian tragacanth specimens, or had been borrowed from the collection of our Institute. The broth culture, filtrated under sterile conditions, of an E. coli-strain used for comparison did not have an embryotoxic effect either.

Hence, according to the investigations, the gramnegative germs, isolated from the different Persian tragacanth specimens, of the genus Enterobacter or their products of metabolism are the cause of the embryotoxic effect of Persian tragacanth after intraperitoneal injection for NMRI mice. Nothing precise can be stated about the nature of these products of metabolism of bacteria. Since, however, the used of the autoclave for 15 minutes at 120 C only weakens, but does not eliminates, the embryotoxic effect of sterile filtrates of broth cultures of Enterobacter, the effective substance must consist at least of a thermolabile and of a thermostable constituents. Whether the thermolabile constituents involves albumins, and the thermostable part possible polysaccharides - like this is known from endotoxins of gram negative germs, must be clarified through further investigations.

Our investigations with decreasing doses of a sterile filtrate of broth culture of enterobacter on the 12th day of gestation show that the number of the dead fetuses decreases in proportion to the dose injected; but that malformations are not increased. Hence it can be concluded that the resorptions and miscarriages, observed in our tests, very probably are no signs of a pure teratogenic action of the products of metabolism of the bacteria.

It is possible that the fetal death is due to massive placenta bleedings, caused by the products of bacteria metabolism of the Enterobacter strains. This might shown by the observation that in some mice, killed immediatly after vaginal bleeding was clinically found, fresh bleedings were detected on the surfaces of the placenta without any fetal residues (state after abortion) and in those with attached fetal sack in which the fetuses in the process of resorption lay. Placenta bleedings, connected with the death of the fetuses, were observed also by THIERSCH (1960) in pregnant rats after injection of lipopolysaccharides, obtained from gram negative bacteria. The cause of the placenta bleedings

could be a disturbance of the blood coagulation, since for dogs, intravenous injections of tragacanth mucilage are deadly on account of the effect exerted on the blood coagulation (WALTON and others, 1959). Such a mechanism could also be implied by the increase of the vascular permeability and lengthening of the time of coagulation found as a result of lipopolysaccharide action, by WESTPHAL and Collaborators (1955).

After repeated subcutaneous injection, a tragacanth mucilage, contaminated with bacteria of the genus *Enterobacter*, as well as the sterile filtrates of broth cultures manufactured from this tragacanth charge and *Enterobacter cloacae* 4034, did not influence the fetal development of the NMRI mice. Hence it must be assumed that the embryotoxic effect, observed after intraperitoneal injection in NMRI mice, of different Persian tragacanth charges, which were contaminated with bacteria of the genus *enterobacter*, and of sterile filtrate of broth cultures of *Enterobacter*, is not due to a systemic effect, as for instance a general disturbance of the blood coagulation, but to a direct action on the uterus and its vascular system.

For the practice, it results from the investigations presented in this study that:

1. Suspensions and emulsions, which can be contaminated by bacteria of the genus *Enterobacter*, should at much as possible not be used in testing the possible teratogenic effect of water insoluble substances on the mouse by means of intraperitoneal injection, and
2. Substances, which cause an increasing of the vascular permeability, or possess considerable vasodilatatory or vasoconstrictory effect, and must be applied parenterally, should not be injected intraperitoneally when testing the teratogenic effect, in order to prevent a fetal injury caused by a local perfusion disturbance of the uterus.

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DIET COMPOSITION AND MINERAL BALANCE IN GUINEA PIGS¹

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The guinea pig is highly susceptible to soft tissue calcification. Wulzen and Bahrs ('41) first reported a stiffness syndrome which has been studied in detail and reviewed by van Wagendonk and Wulzen ('50). The syndrome was characterized by stiff joints and by extensive soft tissue calcification and necrosis. A similar syndrome was reported by Hogan et al. ('50, '54) in guinea pigs that consumed excess phosphorus. Smith et al. ('49) also observed metastatic calcification when certain diets were fed for long periods.

Roine et al. ('49) pointed out that for maximum growth the guinea pig has a high requirement for potassium and magnesium. House and Hogan ('55) observed that high levels of these elements protect guinea pigs from soft tissue calcification. Magnesium deficiency has been implicated in metastatic calcification in other species including the calf (Moore et al., '58), the cotton rat (Constant and Phillips, '54) and the white rat (Tufts and Greenberg, '38). The rabbit has a high requirement for potassium (Hove and Herndon, '55), but part of the requirement can be met by other cations (Wooley and Michelson, '54). The high cation requirement of guinea pigs is partly due to their inability to conserve fixed bases by excreting ammonia in the urine (O'Dell et al., '56). Gum arabic has been reported to be superior to cellulose as a

¹ Approved by the Director of the Missouri Agricultural Experiment Station, Journal Series no. 1727.

source of bulk (Booth et al., '49; House and Hogan, '55), possibly because of its mineral content.

The reluctance of investigators to use the guinea pig for balance studies may have resulted from the fact that the habits of the species are not readily adaptable to the necessary conditions. Nevertheless, it seemed that a study of mineral balances might help explain the superior nature of gum arabic, the injurious nature of high levels of phosphorus, and the ameliorative function of high levels of potassium and magnesium in the diet of the guinea pig. Balance studies of calcium, magnesium, potassium, sodium and phosphorus are reported here.

The balance studies show that a high phosphorus intake results in a negative balance of potassium and magnesium. Although the endogenous fecal excretion of the various minerals was not determined and hence true absorption cannot be calculated, apparent absorption values are reported because they strongly indicate that a high phosphorus level impairs magnesium absorption.

EXPERIMENTAL

Guinea pigs 14 weeks of age or older were used. In general, the animals had received a purified diet from weaning, and in all cases they consumed the respective experimental diets during a three-week preliminary conditioning period. During this period they became adjusted to a small individual cage, to a water bottle, and to a pelleted ration. An additional period of three or 4 days was required for the animals to adjust to the metabolism cage proper.

The metabolism cage was made of galvanized hardware cloth and was small enough to prevent the animals from turning around. The usual size was $9 \times 4 \times 3$ inches and it restricted their movements considerably. Restriction of movement was necessary to prevent coprophagy and to permit accurate collection. The animals were removed from the metabolism cage one hour each day and allowed to exercise in small cages with raised wire bottoms from which quanti-

tative collection could be made. The feces, urine, and refused food were collected daily and fresh food was weighed into the feeder. The dry weight of the food offered as well as that of the food refused was determined and weight of food consumed was determined by difference. Distilled water was supplied ad libitum. Collections were made for a period of 10 days and the daily samples were pooled and refrigerated. The pooled samples of feces were dried in vacuo, weighed, and homogenized before removal of an aliquot for analysis. Two milliliters of concentrated HCl were added to the urine collection bottle daily to serve as a preservative. The fecal receptacle and funnel were rinsed with dilute HCl and distilled water to remove dried urinary salts and the rinsings were combined with the urine sample. Each urine sample was filtered and the total volume determined before removal of an aliquot for analysis.

Phosphorus was determined by a modification of the method of Fiske and Subbarow ('25) after the sample was wet ashed with a mixture of sulfuric, nitric and perchloric acids. In the case of the urine samples it was necessary to remove the excess tin which arose from washing the cage with acid. This was done by treatment with cupferron before ashing. For the other elements, another aliquot was dry ashed at about 550°C. After removal of phosphate ion by use of an ion exchange resin, the calcium and magnesium were determined by the versene titration method of Cheng and Bray ('51). Potassium and sodium were determined by a commonly accepted flame photometric method.

The basal ration was the same as used by O'Dell et al. ('56) and contained acid-washed casein 30; sucrose 47; cellulose 15; soybean oil 4; salts² 4% and vitamin supplements. This diet contained about 0.9% of calcium, 0.4% of phosphorus, 0.5% of potassium, 0.1% of sodium and 0.1% of magnesium. The phosphorus content of the diet was increased by use of the salt mixture of Richardson and Hogan ('46) and by the

²The salt mixture of Hubbell et al. ('37) to which was added 2.5 gm of $\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$ per 100 gm.

TABLE 1
Experimental design and performance of animals. Daily average for a 10-day period

No. 1	RATION				AV. WT. OF ANIMALS		FOOD CON- SUMED	DIGESTI- BILITY OF DRY MATTER	MINERAL CONSUMPTION				
	Approximate composition				Initial of bulk 2	Gain or loss			Ca	Mg	K	Na	P
	P	K	Mg	Type of bulk 2									
	%	%	%		gm	gm		%	mg	mg	mg	mg	mg
33883	0.4	0.5	0.1	GA	513	1.9	21.4	95.0	214	12.9	116	25.7	94.5
33884	0.4	0.5	0.1	C	502	-0.5	22.4	80.2	190	6.7	130	29.1	103.0
33449	0.4	1.5	0.3	GA	538	3.3	21.3	94.8	217	72.3	345	21.3	93.7
33479	0.4	1.5	0.3	C	586	0.05	20.4	81.3	208	67.2	277	22.4	85.5
33514	0.9	0.5	0.1	GA	491	2.1	17.3	92.2	163	13.8	97.0	40.2	154.0
33480	0.9	0.5	0.1	C	457	0.4	18.9	79.5	179	14.2	86.6	47.6	174.0
33516	0.9	1.5	0.3	GA	570	1.4	23.0	93.2	258	78.1	395	59.7	210.0
33515	0.9	1.5	0.3	C	536	2.1	22.2	80.5	206	77.0	306	62.5	206.0
33476	1.8	0.5	0.1	GA	381	0.8	14.7	89.3	147	12.6	70.3	125.0	255.0
33475	1.8	0.5	0.1	C	475	-2.5	21.1	78.2	213	12.7	90.7	181.0	375.0
33478	1.8	1.5	0.3	GA	540	2.3	16.0	94.6	164	57.5	225	132.0	289.0
33477	1.8	1.5	0.3	C	417	2.5	19.6	78.5	200	70.8	276	170.0	351.0

¹ The same as our laboratory number. There were three or 4 animals per group.

² Cellulose (C) or Gum Arabic (GA).

addition of 3.6% $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$. The potassium content was increased by the addition of 2.7% potassium acetate and the magnesium by addition of 0.5% magnesium oxide.

The experimental design and the approximate composition of the diets are shown in table 1. There were 4 diets of low (0.4%), 4 of medium (0.9%) and 4 of high (1.8%) phosphorus content. Within each group two diets had added potassium and magnesium and will be designated as high-potassium and magnesium diets. At each potassium and magnesium level one diet contained cellulflour and the other gum arabic.

RESULTS

The average daily performance of the animals is shown in table 1. Most of the animals were near mature weight and gained slowly or not at all during the 10-day experimental period. The food consumption averaged about 20 gm per day except for the animals on ration 3476 and this was partly due to their smaller initial weight. The daily calcium consumption averaged about 200 mg. The intake of magnesium ranged from 7 to 78 mg, potassium from 70 to 395 mg, sodium from 21 to 181 mg and phosphorus from 86 to 375 mg, varying chiefly because of the composition of the diet. The consumption data were calculated from the average percentage composition determined by at least three analyses.

The average percentage digestibility of the dry matter of gum arabic diets was 93.5% (range, 89 to 95%) and of cellulflour diets 79.8% (range, 78 to 81%). The difference between the average digestibility of the two is 13.5%, a number which is about equal to the percentage of bulk added. One may conclude that cellulflour is poorly digested whereas gum arabic is almost completely digested.

The effect of dietary constituents on the absorption of minerals is summarized in table 2. For brevity of presentation the gum arabic diets were combined and compared with the combined cellulflour diets and the high potassium and magnesium diets compared with the diets of lower content. The terms absorption or apparent absorption as used here

TABLE 2
Apparent absorption of minerals by the adult guinea pig

MINERAL	DIETARY VARIABLES COMPARED						
	Type of bulk		K and Mg level				Phosphorus level
	C (22) ¹	GA (20)	Low (19)	High (23)	0.4% (14)	0.9% (15)	1.8% (13)
Calcium	46 ± 4 ²	58 ± 4	48 ± 4	54 ± 4	70 ± 2**	45 ± 3	37 ± 4**
Magnesium	65 ± 14	76 ± 10	20 ± 71**	78 ± 3**	84 ± 10	77 ± 3	46 ± 23
Potassium	84 ± 2	95 ± 2	81 ± 2	92 ± 1	92 ± 2	90 ± 2	84 ± 3
Sodium	78 ± 3	91 ± 1	80 ± 3	86 ± 3	80 ± 5	83 ± 3	84 ± 3
Phosphorus	43 ± 2	49 ± 4	46 ± 2	45 ± 4	46 ± 4	42 ± 3	48 ± 5

¹ Number of trials averaged is shown within parentheses.

² Standard error of the mean.

** P value less than 0.01 when compared by the Fisher "t" test.

may be defined as 100 minus the percentage of the consumed nutrient recovered in the feces. The absorption of calcium, magnesium, potassium, and sodium was about 10 percentage units greater when gum arabic diets were fed than when cellulose diets were fed. Phosphorus absorption was essentially unaffected.

The higher levels of potassium and magnesium had no effect on the percentage absorption of phosphorus but increased that of calcium, magnesium, potassium and sodium. It seems unlikely that the difference in absorption of calcium and sodium is beyond the limit of experimental error. The percentages of potassium and magnesium absorbed were higher and the absolute amounts absorbed were markedly higher when the high-potassium and magnesium diets were fed. The difference in percentage absorption of magnesium was statistically significant at the 1% level. On the low-magnesium diets, some animals excreted more magnesium in the feces than they consumed.

The absorption of calcium and magnesium on the high level of phosphorus was about 50% of the value observed on the low level of phosphorus. The effect on sodium and phosphorus was of little or no significance. Although not shown in table 2, the average absorption of magnesium on the low-magnesium, high-phosphorus diets was -60% compared to +66% on the medium-, and +42% on the low-phosphorus diets. There was a trend toward lower absorption of potassium as the dietary phosphorus level was increased. Although the percentage difference was not great, the absolute difference was of considerable importance to animals that consumed a low-potassium diet.

The effect of dietary constituents on mineral balance is summarized in table 3. Animals that consumed gum arabic showed a higher retention of calcium and potassium than those on cellulose, but the differences were not statistically significant. High levels of dietary potassium and magnesium increased the retention of these cations and the effect on magnesium retention was significant at the 1% level. The

TABLE 3
Mineral balance of adult guinea pigs
Average balance expressed as milligrams per day per kilogram of body weight

MINERAL	DIETARY VARIABLES COMPARED						
	Type of bulk		K and Mg level		Phosphorus level		
	G (20) ¹	GA (19)	Low (19)	High (20)	0.4% (14)	0.8% (15)	1.8% (10)
Calcium	37 ± 9 ²	54 ± 12	44 ± 10	46 ± 11	65 ± 8	33 ± 13	37 ± 15
Magnesium	-1.5 ± 6	-3 ± 4	-11 ± 6**	6 ± 3**	0.2 ± 4*	9 ± 2*	-23 ± 8*
Potassium	20 ± 10	35 ± 12	20 ± 9	34 ± 13	48 ± 14	30 ± 13*	-6 ± 5*
Sodium	9 ± 6	-3 ± 6	-1 ± 6	7 ± 6	0.8 ± 3	-0.8 ± 6	12 ± 11
Phosphorus	41 ± 9	29 ± 11	37 ± 11	34 ± 9	20 ± 5	38 ± 11	42 ± 23

¹ The number of animals used is shown within parentheses.

² Standard error of the mean.

* P value less than 0.02.

** P value less than 0.01.

effect on the other elements was small and of little significance.

Sodium and phosphorus balances increased as the consumption of these elements increased, but the calcium balance was decreased by high levels of phosphorus. The most significant effect of the high-phosphorus level was to change the magnesium and potassium balance from positive to negative. A major consideration throughout this investigation was to determine when the animals were properly adjusted to the experimental diets. In these trials the animals on the medium-phosphorus diets were in exceptionally good condition and the animals on the low-phosphorus diets were slightly below

TABLE 4

Apparent absorption, pathway of excretion, and balance of minerals in adult guinea pigs fed purified diets¹

MINERAL	APPARENT ABSORPTION	EXCRETED IN THE URINE ²	BALANCE PER KG OF BODY WEIGHT PER DAY
	%	%	mg
Calcium	69	62	65
Magnesium	88	87	7
Potassium	94	93	53
Sodium	78	78	0.2
Phosphorus	44	34	24

¹ Rations 3349 and 3479, 4 animals each.

² Expressed as per cent of total urinary and fecal excretion.

normal in appearance. Consequently, the higher magnesium balance for the medium-phosphorus than for the low-phosphorus diets probably does not represent better nutrition.

From the standpoint of comparative physiology it is of interest to compare the guinea pig with other species as regards mineral metabolism. In table 4 are shown the average values of apparent absorption, pathway of excretion and balance of the various elements in animals fed the most nearly adequate purified diets used in this laboratory. The percentage of apparent absorption of calcium and magnesium is higher for the guinea pig than has been reported for most species. Since apparent absorption is not corrected for the

portion of the element excreted by way of the intestine, it is not possible to tell whether this difference lies in their ability to absorb the element more effectively or in differences in pathway of excretion. Regardless of this consideration, the adult guinea pig absorbs a high percentage of the calcium and magnesium in a purified diet and excretes a similar percentage of the excess elements by way of the urine. The percentage absorption of the other elements is comparable to that observed in other species.

The ratio of calcium to phosphorus retained should be about 1.5 to 1. The ratio observed in these animals was almost 3 to 1. This discrepancy probably arises due to analytical error and to the short duration of the balance study. The retention of potassium is greater than that of sodium as would be expected, but the high retention of potassium may reflect an accumulation of analytical errors.

DISCUSSION

The superiority of gum arabic over cellulose and other sources of bulk reported by Booth et al. ('49) and by House and Hogan ('55) is no doubt due in part to its ash content and in part to the fact that its high digestibility allows more complete absorption of cations. Since gum arabic is at least 90% digested by the guinea pigs, it seems unlikely that its value lies in providing "bulk" in the usual sense of the word. Larrivee and Elvehjem ('54) noted that chinchillas fed gum arabic diets produced far less feces than those fed cellulose. This difference was attributed to constipation in the animals fed gum arabic. Guinea pigs fed gum arabic diets also produce small and scanty fecal pellets, and it seems possible that the explanation in both species is the high digestibility of gum arabic.

It is well recognized that a high calcium content of the diet increases the severity of magnesium deficiency and raises the magnesium requirement, but to the knowledge of the authors the detrimental effect of phosphorus on the absorption of magnesium has not been recognized. When the dietary

potassium and magnesium levels were low, the apparent absorption of magnesium was low and the absorption was decreased further by high levels of phosphorus. It is recognized that the endogenous excretion of magnesium would account for an appreciable amount of the fecal magnesium in the case of the low magnesium diets, but the important point is that high levels of phosphorus decreased the absorption regardless of the magnesium level.

Guinea pigs fed high-phosphorus diets that contain levels of potassium and magnesium commonly fed to other laboratory animals, such as rats and chickens, fail for at least two reasons; there may be other less obvious reasons. They go into a negative potassium balance, because of their inability to conserve fixed bases effectively (O'Dell et al., '56). They also show a negative balance of magnesium which is apparently due to the effect of phosphorus on the absorption of magnesium. There was no evidence for an increased urinary excretion of magnesium on the high-phosphorus diets.

Since a magnesium deficiency is known to cause calcinosis in other species, the soft tissue calcification observed in the guinea pig is probably due largely to a deficiency of this element. A high content of phosphorus in the diet contributes to the syndrome primarily by interfering with magnesium absorption and secondarily by maintaining a high blood phosphorus level (O'Dell et al., '56).

SUMMARY

Guinea pigs near maturity were used to study the effect of type of bulk, and of dietary levels of potassium, magnesium and phosphorus on apparent absorption and retention of calcium, magnesium, potassium, sodium and phosphorus.

The most significant observation was that high dietary levels of phosphorus caused a negative balance of magnesium in guinea pigs that received about 0.1% of magnesium in the diet. The low retention was primarily due to the decreased absorption of magnesium in the presence of excess phosphorus regardless of the magnesium level. A similar though less

marked effect was observed on the absorption and retention of potassium and calcium.

Gum arabic was found to be highly digestible whereas cellulose was essentially indigestible. The absorption of all cations was about 10 percentage points higher from diets that contained gum arabic than from those that contained cellulose.

Guinea pigs absorbed approximately 70% of the calcium and 90% of the magnesium consumed when they were fed a purified diet that contained 0.9% of calcium, 0.3% of magnesium and 0.4% of phosphorus. Large portions of the excreted calcium and magnesium, 62 and 87% respectively, were eliminated by way of the kidneys.

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ON THE IDENTIFICATION BY MEANS OF ELECTROPHORESIS AND
CELLULOSE ACETATE FOIL DYEING OF GELS & THICKENING SUBSTANCES
THAT ARE LEGAL IN SWITZERLAND.

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1. INTRODUCTION

Besides their gelling and thickening properties, gels and thickening substances used in food manufacture or processing also evidence another important characteristic, namely their ability to emulsify and disperse, thereby providing the explanation for their increasingly wide use as stabilizers in the food processing industry.

Article 443 bis of the Federal Food Regulations defines gels and thickening substances as being those capable of yielding aqueous jellies or aqueous, highly viscous solutions already when in low concentrations. (Concerning admissibility of use, see same document, 56, 110, -1956). Even though they are often being used as stabilizers, they are not designated as such in the food regulation. Within the scope of judicial planning concerning food processing in the EEC, namely admissible emulsifiers, stabilizers, gels and thickening substances (WEISS, 1966), stabilizers were defined as such substances suited for maintaining uniform dispersion of two or more immiscible substances. Most gels and thickening substances are included.

Food monitoring efforts have not been wanting to the end of developing practical testing methods for the analysis of gels and thickening substances. Identification is rendered difficult by the fact that these are high-molecular substances for which no easily executed and specific reactions are known, -- except for starch and the alginates. Furthermore they are used in food only in very minute quantities most of the time, because the desired thickening or stabilizing effect often is already achieved for concentrations under 0.5%. The viscometric method (LETZIG, 1934), based on an appreciable viscosity increase of aqueous solutions due to thickening substances, allows an overall indication of any presence of a thickening substance, but does not permit its identification. Various methods are known for qualitative evidence of gels and thickening substances, which among other methods are based on microscopic tests (CZAJA, 1962; BEYTHIEN & DIEMAIR, 1963), on flocculation reactions (LETZIG, 1955) or on paper chromatographic tests of their hydrolysis products (BECKER, 1956; SULSER, 1957; STOLL & PRATT, 1962), that is, of the corresponding monosaccharides. If the gels and thickening substances to be tested are in pure form, they may be easily tested most of the time under the microscope or by the flocculation reaction. If however their presence in food must be shown, in which there is an interfering substance such as egg white material, then the paper chromatographic test of the hydrolysis products is more promising than a flocculation

reaction. However there are cases in which a few gels or thickening substances yield similar sugar components after hydrolysis or where such sugars as glucose and galactose (for instance from lactose in milk products) are detected in the paper chromatographic test, so that the chromatogram will not yield an unambiguous answer. Methods for testing the identity of the purity have been disclosed in the literature (ORGANISATION MONDIALE DE LA SANTE, 1964, 1966). Concerning their numerous applications outside food processing, see also GLICKSMAN (1964).

The process of paper electrophoresis has proved itself for gelatin detection in dairy products (see this publication, 56, 110, 1965). However the cellulose acetate foils are preferable to paper as carrier material, since good separation may be achieved in little time and because the foils may be rapidly rinsed in view of little dye absorption (see section 2.4.1).. We further made use of electrophoresis for the analysis of gels and thickening substances belonging to the polysaccharide group.

The described testing method further allows -- besides identification of gels and thickening substances -- quantitative evaluation by means of dye comparisons with solutions of known concentrations of the pertinent gels and thickening substances, the comparison dealing with color intensity. Our research has shown this simple and easily executed electrophoresis process followed by dyeing promises to be very useful in the analysis of gels and thickening means.

As regards food products, polysaccharides may be determined after removal of fatty and egg-white components by means of alcohol precipitation, which is followed by polysaccharide concentration and the subsequently described method.

2. ELECTROPHORESIS OF GELS AND THICKENING SUBSTANCES

2.1 The cellulose acetate foil as carrier substance

Filter paper is unsuitable for electrophoresis research in gels and thickening substances. When the gels and thickening substances being tested are made visible after electrophoresis-separation (see section 3.3), the filter paper reacts as a polysaccharide when the particular dyeing method is used, and it reacts positively. We tested glass fiber strips and cellulose acetate foils in lieu of filter paper. The latter proved particularly suitable. When testing gelatine too, the paper electrophoresis method proved very useful, and cellulose acetate foils were preferable to filter paper as carrier material, because the lesser dye adsorption allowed rapid rinsing of the foils (see figures 1a and 1b).

An important advantage of cellulose acetate foil electrophoresis consists in the shorter test duration with respect to paper electrophoresis, or about 15 to 30 minutes for micro-electrophoresis (section 2.4.2), so that interferences or secondary effects during separation, such as evaporation of buffer liquid and the resulting undesired concentration increase are only of insignificant magnitude.

2.2 BUFFER SOLUTIONS

The conventional buffer solutions for paper electrophoresis may also be used as conducting electrolytic solutions for cellulose acetate foil electrophoresis. In general, less concentrated solutions are preferred for the latter process. We selected a borate buffer with a pH of 10 for the electrophoresis of the gels and thickening substances of the polysaccharide group, however the sodium-carbonate sodium-hydrogen-carbonate buffer is more suitable when testing gelatine (see section 2.4.3). The buffer solution concentrations are so adjusted that for a terminal potential of 200 volts, a current of less than 1 ma/cm of foil width is generated. Using both buffers demonstrated anodic migration in all gels and thickening substances that were tested, so that they must be coated on the cathode side at the beginning of the test.

2.3 RENDERING GELS AND THICKENING SUBSTANCES VISIBLE.

Following electrophoresis migration, the cellulose acetate foil is removed from the electrophoresis chamber and the position of the gels and thickening substances will be rendered visible by dyeing them. The various gels and thickening substances evidencing different chemical properties, four different dyeing methods are required (see section 3), which may be differentiated through dyeing because the gels and thickening substances migrate at the same rate under electrophoresis.

2.4 RESULTS

2.4.1 COMPARISON BETWEEN CELLULOSE ACETATE FOILS AND PAPER STRIPS

Pherograms I and II in fig. 1a show the results of our electrophoresis test with cellulose acetate foils (4 x 30 cm) in the Elphor-H electrophoresis chamber of GRASSMANN & HANNIG. Except for buffer concentration and the kind of depositing of the substance tested, test conditions are the same as for paper electrophoresis. The amounts of carbonate buffer with pH of 10 and ion size of 0.15 microns are diluted with the same volumes of distilled water. Deposition of substance tested takes place - not with a micropipette - but with a stamp consisting of two parallel platinum laminae absorbing each time 6 microliter of liquid.

It will be noted when comparing figures 1a and 1b that the cellulose acetate foil electrophoresis allows good separation between gelatine and milk eggwhite in much shorter a time than is possible for paper electrophoresis (3 hours in lieu of 14). Furthermore, the foils may be rinsed when dyeing much faster also (20 minutes in lieu of 2 hours).

2.4.2 MICRO-ELECTROPHORESIS OF GELATINE WITH CELLULOSE ACETATE FOILS

The BECKMANN-SPINCO microzone electrophoresis system allows executing simultaneously 8 separations for probe quantities of 0.25 microliter solution of substance, corresponding to 2-10 microgram of the substance tested, on one cellulose acetate foil of 5.5 x 14 cm (see section 4). Deposition of substance tested is performed by means of a microstamp. This allows reducing separation time to 15-30 minutes. Normally the separation time

on a cellulose acetate foil 4 x 30 cm in size and with the Elphor-H electrophoresis chamber of GRASSMANN & HANNIG amounts to about 3 hours (see section 2.4.1)

fig. 1a: electrophoresis on cellulose acetate foils

fig. 1b: paper electrophoresis

Fig. 1a: I=gelatin (from 1% gelatin solution, contains about 60 μ gm gelatin, deposited with micro-stamp)
II= gelatin and milk egg white (isolated from 0.2% gelatin-content yoghurt, deposited with micro-stamp).
= direction of migration (anodic)
test duration: 3 hours; dyeing plus rinsing: 20 minutes

Fig. 1b: I= gelatin (from 1% gelatin solution, micropipette deposition of 10 μ l, contains 100 μ gm gelatin)
II= gelatin and milk egg white (isolated from a 0.2%-content-of-gelatin yoghurt, deposited with stamp).
= direction of migration (anodic)
Test duration: 14 hours;
Dyeing and rinsing time: 2 hours

The pherograms show that yoghurt 1 is free of gelatin and that there is gelatin in yoghurt 2. During gelatin isolation, the milk egg white in yoghurt 2 was completely precipitated by means of heat treatment and removed by means of centrifugal action or of filtration. For gelatin concentration, the filtrate will be raised to 10 fold concentration by vacuum evaporation. If the milk egg white is not completely removed, its egg white bands will appear on the pherogram (see picture of self-made yoghurt (b) and that for yoghurt 1). The pherogram shows remaining egg white still capable of migration in yoghurt 1. For the self-made yoghurt sample, -- yoghurt + gelatin --, one may observe completely denatured milk egg white (remaining at the place of deposition) and capable of migrating.

The pherograms therefore show that gelatine tests in dairy products do not require absolute purity in the separated gelatin. Even if the extract obtained after yoghurt heat treatment and after gelatin concentration does contain some milk egg white, clean separation of egg whites from gelatin by means of electrophoresis is quite possible.

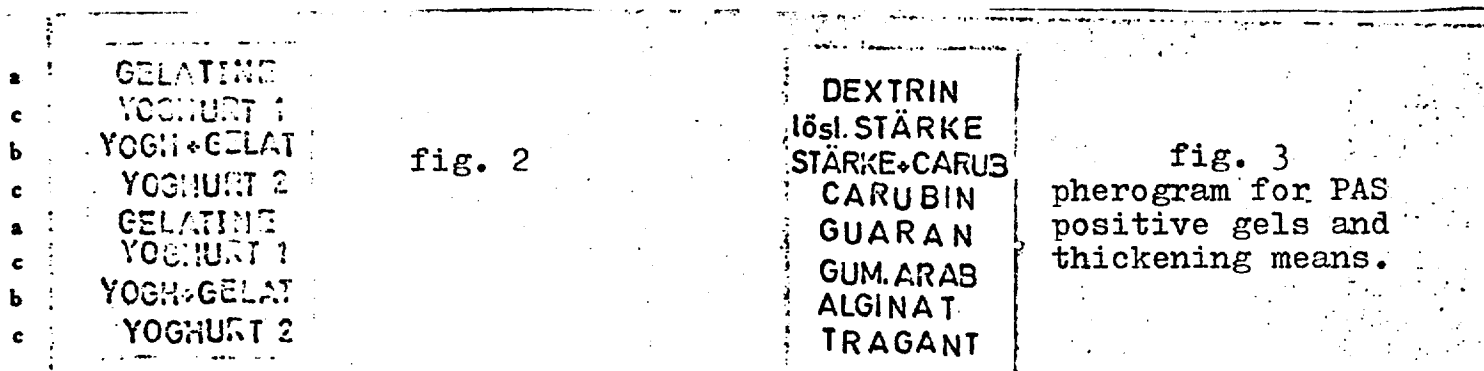


Fig. 2: shows 8 pherograms from double samples deposited by means of micro-stamp

- a) pure gelatin solution as comparison sample; 0.25 ul of 1% solution, corresponding to 2-3 ug of gelatin, deposited
- b) extract from self-made yoghurt containing 0.2% gelatin, 10-fold concentration, 0.25 ul deposited, corresponding to 4-6 ug gelatin
- c) extracts from commercially obtained yoghurt samples (yoghurt 1 and yoghurt 2); same concentration as in b).

TEST CONDITIONS: 200 volts; carbonate buffer of pH 10 and 0.075 micron ion size; anodic migration; separation time of 25 minutes; amido black dyeing (10 B).

Fig. 3: the amounts deposited are 4-5 ugm for dextrin; 2-3 ugm for soluble starch; 7-8 ugm for carubin; 7-8 ugm for guaran; 7-8 ugm for gum arabic; 2-3 ugm for alginate and 2-3 ugm for tragacanth.

TEST CONDITIONS: 200 volts; borate buffer of Ph 10 and ion size of 0.065 microns; anodic migration; separation time of 15 minutes; PAS dyeing.

2.4.3 MICRO-ELECTROPHORESIS OF GELS AND THICKENING MEANS OF THE POLYSACCHARIDE GROUP

We selected borate buffers with a pH of 10 and ion size of 0.065 microns as the conducting electrolytic solution for the electrophoresis of gels and thickening means of the polysaccharide group. Aside from dyeing, the other test conditions are about the same as those for gelatin (section 2.4.2). Section 4 describes execution of dyeing and of micro-electrophoresis. Fig. 3 shows the pherograms of PAS positive gels and thickening means, fig. 5 shows those made visible following tannin preparation and PAS dyeing, and fig. 6 shows those dyed with toluidine blue O. The pherograms of fig. 4 show the results of electrophoresis separation for mixtures from 2 to 4 different PAS positive gels and thickening means. Section 3 will discuss in detail the dye-affinity of the individual gels and thickening means and their classification according to the dyeing method used.

The pherograms show not only that the gels and thickening means tested migrate at different rates, but also that they are sensitive in different degrees to dyeing.

For the test conditions as selected, the alginate and the tragacanth spots are subject to a remarkable though constant curvature (see also fig. 5 and 6) the cause of which is unknown to us. For micro-electrophoresis with borate buffer, gelatin too evidences this effect (fig. 5), but this is not the case when carbonate buffers are used (fig. 2).

In the pherograms shown in fig. 3, carubin, guaran and gum arabic evidence nearly the same migration rate. If a longer separation time is used, namely 25 minutes, -fig. 4 -, in lieu of 15 minutes, they may be separated from one another.

If the pherograms of figures 5 and 6 are more closely examined, it will be observed that several gels and thickening means migrate nearly at the same rate. Agar-agar, carubin, and tragacanth in fig. 5, carrageen, sodium pectate and alginate in fig. 6 therefore cannot be separated from one another by means of electrophoresis under the given test conditions. In order to identify them, an improved separation may be achieved as in the case of the PAS positive gels and thickening means (fig. 4) by a longer separation time of 20-30 minutes, and they may be further differentiated because of their variable dye-ability (see table 1, section 3).

DEXTRIN
DEXTR+CARUB+GUAR+
CARUBIN G.ARAB
DEXTR+CARUB+GARAB
GUARAN
DEXTR+CARUB+GUAR
GUMMI ARABICUM
GUARAN+G.ARAB

Figure 4
Electrophoresis-separation of mixtures
of PAS positive gels and
thickening means.

Deposited amount for dextrin was somewhat less (2-3 micrograms in lieu of 4-5), amounts for carubin and guaran were somewhat more (9-10 microgms in lieu of 7-8) than was the case for pherograms in fig. 3. Longer test duration was required for their separation (25 minutes in lieu of 15). Test conditions:

terminal potential: 200 volts
borate buffer: pH = 10; ion size: 0.065 microns
anodic migration
separation time: 25 minutes
PAS dyeing

AGAR-AGAR
AGAR+G.ARAB
GUMMI ARAB
CARUBIN
M.CELL+CARUBIN
METH.CELL
TRAGANT
GELATINE

Fig. 5

Pherograms of gels and thickening means rendered visible by means of tannin and PAS dyes preparation.

The deposited amount for agar-agar is 4-5 microgm, for gum arabic 7-8 microgm, for carubin 8-10 microgm, for methyl cellulose 4-5 microgm, for tragacanth 4-5 microgm, and for gelatin 2- microgm. Test conditions:

terminal potential: 200 volts

borate buffer pH: 10; ion size: 0.065 microns

anodic migration

separation time: 15 minutes

tannin and PAS dyeing preparation*

SUMMARY

The analysis process described enables identification of individual gels and thickening means as such. In the OFFICIAL METHODS OF ANALYSIS of the AOAC (1965) is listed a method allowing separation of gels and thickening means in food products such as ice cream and mayonnaise after removal of fat and egg white components by means of alcohol precipitation. If this precipitation reaction is positive, then electrophoresis and our dyeing method will allow precise identification of the alcohol precipitate.

Quantitative evaluation may take place by comparing with the color intensities of solutions of known concentrations of the particular gels and thickening means. Coarse estimates may be made by glance alone, photometry being appropriate for precise measurements.

Fig. 6
Pherograms of gels and thickening means with affinity for
toluidine blue O

AGAR-AGAR
CARBM-CELL
CARRAGEEN
G.A.RAD-PEKT
PEKTAT
GUM. ARAB
ALGINAT
TRAGANT

The amounts deposited are, for agar-agar, 4-5 ugm; for carboxy methyl cellulose, 4-5 ugm; for caarageen, 2-3 ugm; for pectate, 4-5 ugm; for gum arabic, 7-8 ugm; for alginate, 2-3 ugm; and for tragacanth, 4-5 ugm.

Test conditions:

terminal potential: 200 volts

borate buffer pH: 10; ion size: 0.065 microns.

anodic migration

separation time: 15 minutes

dyeing: toluidine blue O

3. DYEING OF GELS AND THICKENING MEANS ON CELLULOSE ACETATE FOILS

3.1 FIXING

Prior to dyeing, the gels and thickening means deposited on the cellulose acetate foils should be first fixed so as not to be dissolved during the dyeing process in the dyeing bath. As other egg white bodies, gelatin may be denatured by drying between 80 and 100°C and therefore be fixed, but this is not recommended for cellulose acetate foils, unfortunately, because of the wear on the foil. We make use of a fixing bath instead of the drying process, such bath consisting of a solution of trichloroacetic acid for gelatin and of ethanol for the gels and thickening means of the polysaccharide group.

3.2 GELATIN DYEING

Dyeing of gelatin, which belongs to the protein group, occurs with the conventional egg white substance amido black 10 B, an acid azo-dye. The dyeing process is based on salt formation of the dye's acid groups with the free groups from the egg white. For gels and thickening means from the polysaccharide groups, dyeing must be undertaken in another manner, because there are no free amino groups and therefore do not respond to amido black 10 B (see fig. 7 and table 1).

3.3 PAS DYEING

The PAS (PERIODIC ACID SCHIFF) reaction, recommended by HOTCHKISS (1948) for the dyeing of polysaccharides in animal and plant tissue preparations used in histology, was carried over on our part to gels and thickening means. It was found that not all gels and thickening means could be dyed in this manner. Therefore we make a distinction between PAS positive and PAS negative gels and thickening means.

The Schiff reagency used in PAS dyeing consists of a colorless solution of fuchsin-sulfur acid prepared from adding potassium pyrosulfite and hydrochloric acid to a red, aqueous fuchsin solution. The released sulfur dioxide reduces the red fuchsin to a colorless leuco compound. This Schiff reagency is a known reagency test for aldehyde groups. The gels and thickening means to be dyed first are allowed to react with periodic acid for the generation of aldehyde groups. Two neighboring hydroxyl groups in the polysaccharide molecule will be oxidized to aldehyde groups in the presence of C-C fission. These aldehyde groups react with the Schiff reagency and generate a lilac-red color. Carubin, guaran, soluble starch, dextrin, alginate, tragacanth and gum arabic belong to the PAS positive gels and thickening means. Sodium pectate and carrageen are slightly PAS positive, carrageen's color being very slow to appear.

Polysaccharides, methyl cellulose, cellulose acetates and agar-agar, that lack neighboring hydroxyl groups, correspondingly are PAS negative. Water soluble methyl cellulose shows an average substitution index of 1.64 - 1.92 (GLICKSMAN, 1963) and also contains methyloxyl groups not only as regards the carbon atom C-6 but also to some extent the carbon atoms C-2 and C-3.

For negative PAS and slightly positive PAS gels and thickening means, we found two other ways of dyeing:

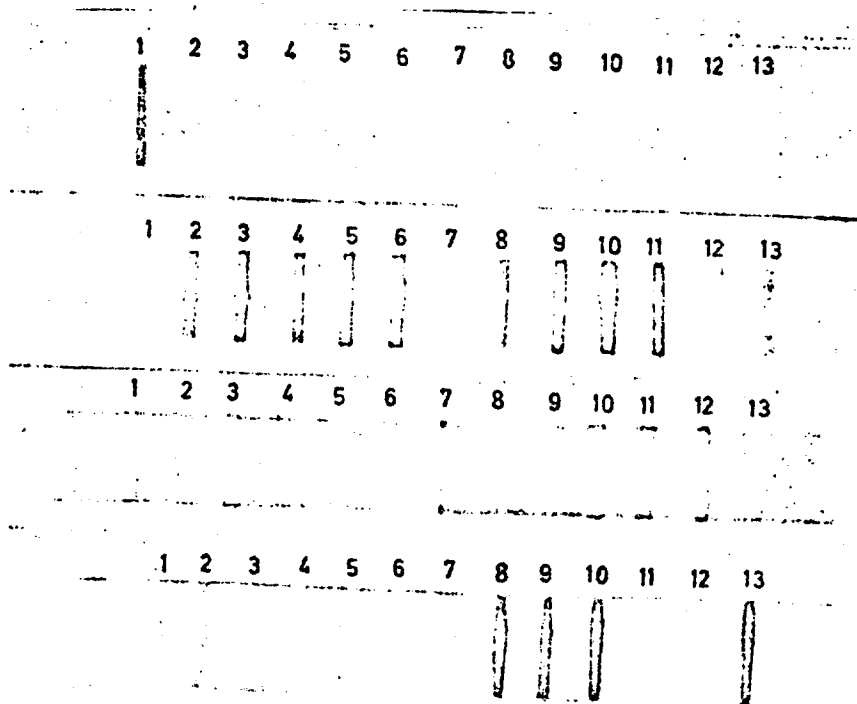
1. a prior treatment with tannin and subsequent PAS dyeing,
2. dyeing with toluidine blue O.

3.4 TANNIN TREATMENT AND PAS DYEING

We first considered using tannin for fixing the gels and thickening means. It was then shown that following such treatment, methyl cellulose could be dyed with the PAS reaction. Previously we had spent much time in vain looking for a way of dyeing methyl cellulose. Besides the latter, gelatin, carubin, agar-agar, tragacanth and gum arabic may also be rendered visible in this fashion. The tannin reaction occurring during this dyeing process -- if so -- is not known to us, except for the corrosive effect.

Besides tannin, we tested many other precipitation means for the polysaccharides, such as lead acetate, phosphor tungsten acid, mercuric chloride and barium chloride, though without satisfactory results obtaining. It was solely when pre-treating with barium chloride that better dyeing of carboxymethylcellulose was achieved. However toluidine blue O provides more sensitive dyeing.

Fig. 7
Dyeing of gels and thickening means on cellulose acetate foils



Numbers 1 through 13 refer to the gels and thickening means in table 1 below. In this dyeing test, the samples were deposited by means of a stamp and about 1.5 cm apart on a membrane supported from a frame; membrane size was 2.5 x 30 cm. Each amount deposited contains about 6 ul of a 1% solution, that is, about 60 ugm of the particular gel or thickening means. Execution of dyeing is described in section 4.4.

3.5 DYEING WITH TOLUIDINE BLUE 0

Toluidine blue 0 is a thiazine dye used in histochemistry for dyeing acid mucopolysaccharides such as heparin and chondroitin sulphates (RIENITS, 1953). Polysaccharide affinity for toluidine Blue 0 assumes the presence of acid groups in the molecule. Those gels and thickening means that may be dyed with toluidine blue 0, that is, sodium pectate, alginate, tragacanth, gum arabic, carboxymethylcellulose and carrageen, contain either uronic acids or sulfate groups in the molecule.

3.6 RESULTS

By making use of the four different dyeing processes, all gels and thickening means admitted under article 443 bis of the Swiss Food Regulations may be dyed differently on cellulose acetate foils (fig. 7 and table 1).

Table 1
Affinity of gels and thickening means on cellulose acetate foils

Gel- und Verdickungsmittel	Amido- schwarz fult	PAS- Anfärbung	Gumm- behandlung + PAS- Anfärbung	Toluidin- Blau O.
1. Gelatine	---	---	(+)	---
2. Na-Pektat	---	(-)	---	(+)
3. Carubin* **	---	---	---	---
4. Guar* **	---	---	---	---
5. Lösliche Stärke	---	---	---	---
6. Dextrin	---	---	---	---
7. Agar-Agar	---	---	---	---
8. Carrageen	---	---	(+)	---
9. Alginat	---	---	---	---
10. Tragant	---	---	---	---
11. Gummi Arabicum	---	---	---	---
12. Methyl-Cellulose	---	---	---	---
13. Carboxy-Methyl- Cellulose	---	(-)	---	---

++ high affinity
+ good affinity

(+) slight affinity
- no affinity

From the dyeing results for the gels and thickening means tested and listed in fig. 7, a listing is made in table 1 where the dyeing methods of the four different kinds used are referred to by plus or minus signs for easier visualisation.

4. PROCEDURE

4.1 SPECIAL EQUIPMENT

BECKMAN-SPINCO microzone electrophoresis system***,
consisting of:
microelectrophoresis cell
micro-sample depositing stamp (0.25 ul sample)
cellulose acetate foil (also designated as membrane), 5.5 x 14 cm
for 8 samples at a time
power supply (potential: 0-500 volts, 0-50 ma)

FOR ELECTROPHORESIS ON LARGE CELLULOSE ACETATE FOILS:

Elphor-H electrophoresis chamber of GRASSMANN & HANNIG, with
associated rectifier and regulating transformer (Bender & Hobein,
Munich, Zurich)
membranes of 4 x 30 cm (Schleicher & Schuell, AG, Feldmeilen)
sample depositing-stamp, about 6 ul sample (Kontron AG, Zurich)

REAGENTS:

borate buffer (pH=10, ion size = 0.13 microns): 12.37 gm
(= 0.2 mol) boric acid in 100 ml 1-n NaOH solution, completed with
distilled water to 1 liter. Then mix 600 ml of this solution with
400 ml of 0.1-m NaOH.

sodium carbonate, sodium hydrogen carbonate buffer (pH=10, ion size = 0.15 microns): mix 750 ml 1-m sodium carbonate solution with 750 ml of 0.1-m sodium hydrogen carbonate solution and with 500 ml distilled water.

PERIODIC ACID SOLUTION: 2 gm periodic acid in 10 ml distilled water solution, then mix with 90 ml 96% vol. ethanol.

TRICHLORACETIC ACID: 5% aqueous solution

TANNIN SOLUTION: 10% aqueous solution *****

SATURATED AMIDO BLACK 10 B SOLUTION: about 0.1 gm amido black in a mixture of 9 parts volume methanol and 1 part volume glacial acetic acid, dissolution through repeated shaking. Solution must be filtered prior to use.

SCHIFF'S REAGENT: 1 gm fuchsin in 100 ml distilled water, hot dissolution, cooling to about 50°C; mixing with 1 ml concentrated hydrochloric acid and 2 gm potassium pyrosulfite, hard shaking, rest over night. Shaking with about 1gm charcoal prior to use and filtering.

TOLUIDIN O SOLUTION: 0.2% aqueous solution

formaldehyde-AMMONIA MIXTURE: mix 1-m formaldehyde solution with 1-m ammonia hydroxide solution in equal parts volume.

ALCOHOL-HYDROCHLORIC ACID MIXTURE: mix 1 part volume 1-n hydrochloric acid with 2 parts volume denatured alcohol (aceton spirit).

METHANOL - GLACIAL ACETIC ACID MIXTURE: 1 part volume glacial acetic acid with 9 parts volume methanol

TRANSPARENCY SOLUTION: 1 part volume glacial acetic acid and 3 parts volume methanol (always fresh).

methanol-SOLUTIONS OF TESTED GELS AND THICKENING MEANS: 1% solution of individual gels and thickening means in the borate buffer. Exception: a concentration of only 0.3% for agar-agar, because a 1% solution may already cause gel binding. As regards carubin, guaran and tragacanth, only about 20% of the substance in borate buffer goes into solution.

4.3 EXECUTION OF MICRO-ELECTROPHORESIS

4.3.1 FILLING THE MICRO-ELECTROPHORESIS CELL WITH BUFFER SOLUTION

The micro-electrophoresis cell consists of the buffer containers, of the electrode chambers, of a support frame for tensioning and a membrane, of a cell-cover and a cell upper part with slits and grooves for the micro-depositing stamp when depositing samples. Upon removing the cell cover, the cell upper part and the support frame, the siphon located between the electrode chambers will be made horizontal by means of one finger and the cell will be filled through the siphon opening to a height between the lines FLUID LEVEL with buffer solution. When testing gels and thickening means of the polysaccharide group, borate buffer with a pH of 10 and ion size of 0.065 microns is used, and for the gelatin test, a buffer of sodium carbonate / sodium hydrogen carbonate with a pH of 10 and ion size of 0.075 microns. The buffer solutions described in section 4.2 to that end will be first diluted with distilled water in equal parts volume. Following filling of the micro-electrophoresis cell, buffer drops on the cell wall above the buffer level are carefully removed by means of filter paper in order to avoid secondary contacts of electrical current beyond the membrane.

4.3.2 EMPLACING THE MEMBRANE

The membrane first is slightly wetted with the non-diluted buffer being used (see section 4.2). In order to wet evenly, the membrane is placed flat on the surface of the buffer solution. After wetting, the foil is dipped into the buffer solution by means of tweezers. White air inclusion spots are thus avoided. The wet membrane then is removed and easily compressed between two sheets of thick filter paper.

Next the evenly wetted membrane is so tensioned in the supporting frame that all the pegs of the latter fit into the membrane's holes, and therefore the membrane will be held equally tautly everywhere. The supporting frame with tensioned membrane will be so inserted in the micro-electrophoresis cell that the reference hole of the foil will precisely coincide with the numeral 1 of the numbers marked on the cell upper part. This aids remembering the sequence of the deposited samples.

4.3.3 SAMPLE DEPOSITION

After emplacing the cell upper part, deposition of samples may begin. By means of a glass rod, a drop of the solution to be tested is put on a glass plate that is kept neat. The micro-sample depositing stamp by means of its platinum laminae touches the sample drop and the liquid film so created is deposited on the membrane, the platinum laminae remaining about two seconds in touch with the membrane. Prior to the next sample deposition, the platinum laminae of the micro stamp will be rinsed with distilled water and dried by means of careful dabbing with filter paper. Once all eight samples have been deposited, the cell cover is put into position and electrophoresis migration may begin.

4.3.4 ELECTROPHORESIS MIGRATION

The micro-electrophoresis cell is so connected to the power supply that the location of sample deposition is on the side of the cathode. Sample migration is toward the anode. Terminal potential is set to 200 volts. For the buffer concentration used, the current density does not exceed 1 ma/cm of foil width (= 5.5 ma), which is a good value for the migration and separation of the samples that were tested. Upon termination of the desired test duration, which may range from 15 to 30 minutes, the current is shut off, the electrophoresis cell is opened, the membrane is removed and then dyed. Dyeing takes place as described in section 4.4.

4.4 DYEING OF GELS AND THICKENING MEANS

The four different dyeing methods will be discussed in the sections below.

4.4.1 AMIDO BLACK 10 B DYEING

This method is used for rendering visible gelatin besides other egg white bodies. In order to fix the egg whites, the membranes will be immersed for 2-3 minutes in 5% trichloroacetic acid solution. Then dyeing takes place for 10 minutes in the saturated amido black 10 B solution. Bleaching of that part of the membrane not carrying egg white takes place when the foil is immersed in a mixture of methanol and glacial acetic acid. The solution will be decanted after 2-3 minutes and replaced by a fresh one. Following threefold washing, white foils are obtained, on which gelatin spots and possibly others appear dyed deep-blue. The foil is rendered transparent in accordance with section 4.5.

4.4.2 PAS DYEING

This method is used to render visible carubin, guaran, soluble starch, dextrin, alginate, tragacanth and gum arabic. The membrane is inserted for 5 minutes in a periodic acid solution. This results in oxidation and also simultaneously in fixing by means of the alcohol of the periodic acid solution. Thereafter the membrane is immersed for 10 minutes in Schiff's reagent so that the individual spots of the tested gels and thickening means appear dyed lilac-reddish. In order to remove the excess Schiff reagent, the membrane is placed for one minute in a mixture of formaldehyde and ammonium, so that solution and membrane become intensely red because of the released fuchsin. In order to bleach that part of the foil free of gels and thickening means, the foil will be rinsed first with denatured alcohol and then several times with the mixture of alcohol and hydrochloric acid. The colortone of gels' and thickening means' spots changes from lilac-red to red-violet. The foil is rendered transparent in accordance with section 4.5

4.4.3 PRE-TREATMENT WITH TANNIN AND PAS-DYEING

This method is mostly used for rendering visible methyl cellulose and agar-agar. Other gels and thickening means that may also be dyed in this manner are gelatin, carubin, tragacanth and gum arabic. The membrane is placed for 7-8 minutes in the 10% tannin solution and then for 5 minutes in the periodic acid solution. Upon periodic acid treatment, foil and solution turn brown. The brown solution is decanted and replaced by new periodic acid. Dyeing proceeds as described in section 4.4.2

4.4.4 TOLUIDINE BLUE O DYEING

This method is mostly used for rendering visible carrageen, carboxymethylcellulose and sodium pectate. Other gels and thickening means that may be dyed with toluidine blue O are alginate, tragacanth and gum arabic. The membrane is placed for 10 minutes in the dye solution. Then the major part of the excess dye is removed by compressing the dyed foil between two sheets of filter paper. Following rinsing with ordinary tap water, the membrane is air dried.

4.5 RENDERING FOILS TRANSPARENT FOR PHOTOMETRIC EVALUATION

Following dyeing, the membrane is immersed for 2-3 minutes in methanol. Then it is placed on a glass plate or disc and subjected for 30 seconds to a fresh mixture of 3 parts volume methanol and 1 part volume glacial acetic acid. Glass plate and membrane are removed from the methanol / glacial acetic acid bath. Upon removing the excess liquid with a rubber sponge or filter paper, they are dried at 110°C for five minutes in an oven. The now transparent pherograms may be kept on the glass plates. They may be carefully removed from the glass plates for the purpose of photometric evaluation and they may be placed in glassines or the likes. The foils treated with toluidine blue O are not subjected to the methanol / glacial acetic acid bath because the spots of the tested gels and thickening means would dissolve, except for carrageen.

Gratitude is expressed to Herr Wenger, chemist from Bern, for verification of this work.

* Only a part hardly visible in this reproduction of the deposited agar-agar migrates on the cellulose acetate foil because of electrophoresis. The component initially remaining is more visible. If dissolved in 0.1-n hydrochloric acid and subsequently neutralized, agar-agar lends itself to easier deposition and will be rendered more easily visible than when in a buffer solution. It was also further shown that for pre-treating agar-agar with tannin, a 0.1% alcohol tannin solution is better suited than the previously used 10% aqueous tannin solution.

** Carubin and guaran dissolve only to 16-19% in borate buffer. The insoluble part was subjected to centrifugation. Depending on quality, dye affinity of carubin will be more or less pronounced. It is strongest for carboxymethyl-carubin; it is very slight for carubin-fleur. Affinity as a function of quality and origin is being tested for further commercial products.

*** Representative: Kontron AG, Zurich

**** A 0.1% alcohol solution gave better results in more recent tests.